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EVALUATION OF THE FERTILIZATION PROPERTIES OF ALGAL BIOMASS AND ASSESSMENT OF KOH-INDUCED FLOCCULATION OF PW95 ALGAL CELLS

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EVALUATION OF THE FERTILIZATION PROPERTIES OF ALGAL BIOMASS AND ASSESSMENT OF KOH-INDUCED FLOCCULATION OF *PW95* ALGAL CELLS

by

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Abstract

Microalgae from the Coal Bed Methane (CBM) ponds of the Powder River Basin (PRB) in Southeastern Montana have the potential to be utilized as fertilizer for use on the economically important plants of Montana. Three very important economic field crops of Montana (winter wheat - *Triticum aestivum*, potato - *Solanum tuberosum,* and flax - *Linum usitatissimum*) were used for the fertilization experiments. Isolates of unicellular green algae - *PW95* (sequenced as *Neospongiococcum sp*.) from the CBM ponds, and blue-green microalgae – *Cyanobacteria* (*Anabaena Cylindrica*) were cultured in the laboratory and the cells were concentrated using gravity sedimentation. Nutrient analysis of the *PW95* cultures showed nitrogen as the most abundant component with a concentration of 1240 mg/L. Other components, such as potassium and phosphorus, 264 mg/L and 130 mg/L respectively, were also detected. Concentrated algal slurry was added to the seedlings after the determination of their nutrient composition and the wheat and potatoes were harvested after 120 and 100 days respectively.

Overall, when compared to control wheat grown with only water, or with water and a commercially available fertilizer, the *PW95*-fertilized wheat had higher chlorophyll content, more tillers (side shoots), and higher ratio of inflorescences (groups of flowers) per stem. Data analysis showed a statistical difference in plant height of wheat fertilized by *PW95*. In terms of harvest, the average total dry weight for PW95-fertilized wheat was 117% and 47% more than those of water and chemical fertilizer (Miracle-Gro - M.Gro) - treated wheats respectively. Measurements of the seed weights showed that *PW95*-treated plants are 123% and 58% higher than corresponding measurements for wheat treated with water and M.Gro respectively. The results of this study suggest that *PW95* from the CBM ponds may be a viable source of fertilizer for crops and other economically important plants of Montana and may contribute to the development of an economically important and locally obtainable product from the ponds. These results were not as pronounced in *PW95*-fertilized potatoes.

A major bottleneck to effective implementation and deployment of microalgae as a fertilizing agent is the availability of biomass which originates from noticed deficiencies in biomass harvesting techniques. Experiments conducted using KOH as a flocculating agent for biomass harvesting showed that *PW95* cells agglomerate as the pH of the suspension increases. An optimal pH level was found to be 11.5. Highest flocculation efficiencies of 28% and 42% were achieved at optimum pH 11.5 over a settling time of 15 and 30 minutes respectively. However, efficiency at pH 12 (51.82%) was marginally higher (3.4%) than the efficiency at pH 11.5 at 45 minutes.

As widely stated in literature, the use of KOH as flocculant is intended to contaminate, with potassium, the biomass product and lower its quality. However, with potassium being an essential growth agent for most crops, the biomass product from the flocculation experiment could represent an innovative method of increasing the properties and performance of *PW95* as a biofertilizer.

Keywords: Microalgae, Biomass harvesting, Flocculation, Biofertilizer, Cold Bed Methane (CBM) produced water

Dedication

To God: For the gift of life, and unending mercies.

To my parents: I appreciate their unwavering love and the many sacrifices they made in ensuring that I have better opportunities than were available to them. I love you so much.

To my siblings: You have been the best team of cheerleaders anyone could ever ask for. Thanks for supporting me on this journey.

To my friends: Without your friendship, my time at Montana Tech would have been quite lonely.

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1. Introduction

Microalgae are unicellular photosynthetic microorganisms which can be found nearly everywhere. They have diverse biological characteristics. All microalgae are classified as either prokaryotes - cyanobacteria, which are not *sensu stricto* algae, or eukaryotes - green algae and the diatoms (Li *et al.* 2008a). Microalgae are known for their high growth rates and photosynthetic efficiencies because of their simple structures. Demirbaş (2006) estimated that microalgal biomass production is up to 50 times faster than that of switchgrass - the fastest growing terrestrial plant. The main requirements for their growth include sunlight, nutrients, and adequate aeration (Aslan & Kapdan, 2006). In terms of variety, more than 50,000 species of microalgae have been estimated to exist and only about 60% of these have been investigated (Mata *et al.* 2010).

Some species of microalgae were discovered in the coal bed methane (CBM) fields where methane gas is extracted in the Powder River Basin (PRB) located in northeast Wyoming and southeast Montana. The PRB is well known for its huge coal and viable natural gas deposits (Bartos & Ogle, 2002). According to the Wyoming Oil and Gas Conservation Commission (WGOCC), the amount of produced water from CBM production in the Wyoming portion of the PRB alone was 171 million barrels (5.4 billion gallons) in 2016. Figure 1 (adapted from http://wogcc.state.wy.us/coalbedchart.cfm) shows the CBM water production by month in 2016 in the PRB. This volume of water coupled with its chemical constituents and specifically its high sodium content poses threats to the environment if discharged to surface water directly (ALL, 2003). Hence, produced water from the CBM operations in the PRB are usually impounded in lined ponds. Despite the chemical constituents, some algal lifeforms have been observed in the

ponds. Partial DNA sequence analyses (18S and 16S) of an isolated alga from the CBM ponds indicated a unicellular green alga, *PW95*, of the *Chlorococcaceae* family (Hodgskiss *et al.* 2016).

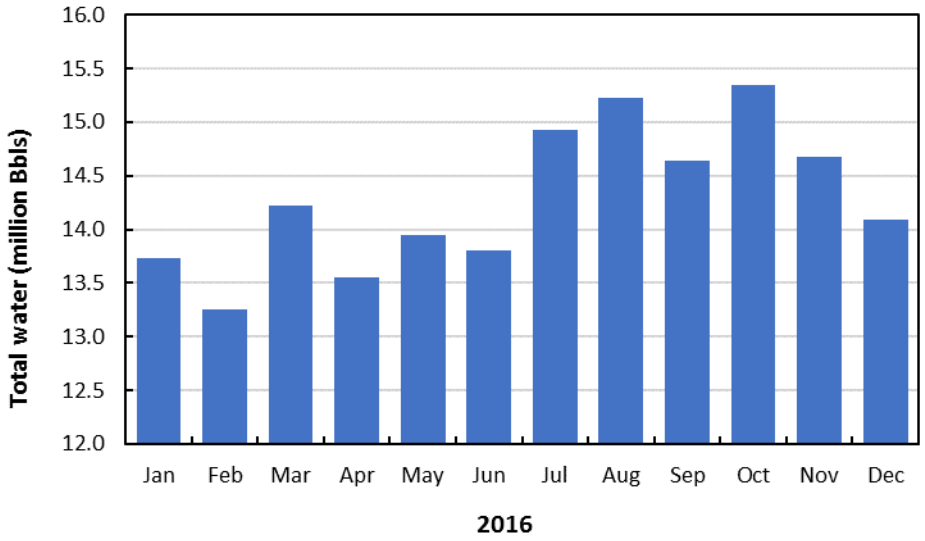

Figure 1: 2016 CBM water production by month in Wyoming

Various species of microalgae have been and are being cultivated for their varying high-value derivatives. Extensive studies have been conducted on the use of microalgae as a useful feedstock for renewable fuels such as biodiesel and ethanol. Microalgal extracts have also found applications in the aquaculture market, production of human and animal food, and cosmetics (Spolaore *et al.* 2006). Analysis of microalgal cells shows they are rich in nutritional constituents such as proteins, lipids, and carbohydrates. In some species, the concentration of these constituents is higher than those found in some human food sources (Table I). Apart from being incorporated into human nutrition and livestock feed, microalgae are being used in organic farming as biofertilizers due to their high contents of nitrogen, phosphorus, trace elements, and growth hormones which are required by plants (Spolaore *et al.* 2006; Thirumaran *et al.* 2009). Consequently, their applications are known to increase soil's nutrients and water-binding capacity, enhance the production of antibiotics and facilitate the biodegradation of organic matter

in the soil (Critchley & Ohno 1998; Bhardwaj *et al.* 2014). The use of algae as fertilizer can be traced back to the common use of seaweeds by ancient European and Asian farmers (Thirumaran *et al.* 2009). In present day world, advancement in technology and research of algal chemical constituents have validated their use for crop fertilization.

Table I: Composition of food sources and algae - % dry matter (Becker, 2004)

Commodity	Protein	Carbo-hydrate	Lipid
Bakers' yeast	39	38	1
Meat	43	1	34
Milk	26	38	28
Rice	8	77	2
Soybean	37	30	20
Anabaena cylindrica	43–56	25–30	4–7
Chlamydomonas rheinhardii	48	17	21
Chlorella vulgaris	51–58	12–17	14–22
Dunaliella salina	57	32	6
Porphyridium cruentum	28–39	40–57	9–14
Scenedesmus obliquus	50–56	10–17	12–14
Spirulina maxima	60–71	13–16	6–7
Synechococcus sp.	63	15	11

In despite of these numerous potentials, the cost associated with the biomass harvesting from culture media makes up to about one-third of the total production cost (Grima *et al.* 2003) which involves energy intensive harvesting techniques (Norsker *et al.* 2011; Draaisma *et al.* 2013). Likewise, even with the extensive research on microalgae over the years, no harvesting technique has been found to be well suited and economical for all microalgal species (Mata *et al.* 2010).

When deployed alone, most of the well-known solid-liquid harvesting techniques such as sedimentation, centrifugation, filtration, and floatation have proven to be inefficient or uneconomical. This difficulty is related to microalgae's diverse morphology which is species-specific, and the desired quality of the biomass (Milledge & Heaven, 2013). Therefore, an

efficient harvesting technique is species-specific; it depends on the properties of the culture medium and the quality of the product (Olaizola, 2003; Richmond, 2008; González-Fernández & Ballesteros, 2013). Hence, recent approaches combine the use of two or more harvesting techniques. Such approaches often involve the use of one technique for pre-concentration of the microalgae prior to further dewatering by another technique (Brennan & Owende, 2010).

Of the well-known conventional harvesting methods, flocculation has been considered as a cost-effective, environmentally friendly, and superior harvesting technique because it is less sensitive to microalgal species variation and it allows the treatment of large quantities of microalgal culture (Pushparaj *et al.* 1993; Lee *et al.* 1998; Vandamme *et al.* 2013). Flocculation can be initiated with the addition of flocculating agent(s) to the microalgal suspension, altering the medium's growth conditions, or with the use of naturally flocculating microalgae (Divakaran & Pillai, 2002; Uduman *et al.* 2010). The addition of a flocculating agent reduces or shields the negative charge of the microalgal cells (McGarry, 1970; Lee *et al*. 1998; Papazi *et al.* 2010). Studies have shown that microalgal cells will aggregate at high pH due to the precipitation of $CaCO_3$ and $Mg(OH)_2$ (Ayoub *et al.* 1986; Semerjian & Ayoub, 2003). Overall, flocculation efficiency is dependent on the processing conditions comprising culture pH and ionic strength, presence of extracellular organic material (EOM), culture age, concentration and type of flocculant, and cell density. In practice, flocculation is often used as a pre-concentration step in increasing the effective particle size of the microalgal cells. After forming aggregated mass, the flocs are further dewatered using other techniques such as sedimentation, filtration, and centrifugation (Grima *et al.* 2003). This combined approach represents the most promising cost and energy efficient alternative (Salim *et al.* 2011). Uduman *et al.* (2010) stated that the flocculation concentration factor could be as high as 200-fold with algal slurries yielding 1-7%

total suspended solids (TSS) by mass. Likewise, further dewatering of pre-concentrated algal slurries could achieve an algal paste of 18-25% TSS.

1.1. Objectives

The main focus of this work is to evaluate ways of stimulating Montana's coal bed methane in an environmentally sound manner. The specific objectives are as follows:

- Evaluate the viability of microalgal biomass in fertilizing economically important crops in Montana.
- Assess algal biomass for macro- and micro-nutrient composition.
- Evaluate the effectiveness of KOH-induced flocculation in harvesting *PW95*.

The last objective was noted as a potential obstacle to the achievement of this work due to the difficulty in concentrating microalgal. Hence, concentration experiments were conducted by increasing the pH of the medium through the addition of potassium hydroxide (KOH). Various studies have shown that induced flocculation of cells by an alkali is optimum within a pH range of 9-12 (Blanchemain & Grizeau, 1999; Ras *et al.* 2011; Spilling *et al.* 2011; Huo *et al.* 2014). The choice of KOH as a flocculant would result in algal biomass that is contaminated with potassium. However, the excess potassium boosts the fertilizing properties of the biomass.

1.2. Thesis Outline

Detailed background information on the source, morphology, and use of microalgae, CBM produced water and ponds as well as the merits and demerits of the common solid-liquid separation techniques are discussed in Chapter 2. The $1.2 million "Energy Policy Goals" research project funded by the Montana Board of Regents is aimed, in part, at testing microalgae fertilization potentials for economic crops of Montana are also discussed in Chapter 2. Since biomass availability is affected by separation efficiency, different techniques for harvesting

microalgae is reviewed in Chapter 2. For this work, the harvesting method deployed focuses on inducing flocculation via pH increase through KOH addition. Likewise, the advantages and disadvantages of microalgal flocculation over other separation methods were equally explored. The overall steps taking in culturing, harvesting, and biomass application to crops are enumerated in Chapter 3. In Chapter 4, the overall results of this thesis is evaluated based on the analysis of the results from the fertilization, and harvesting experiments. Finally, Chapter 5 includes discussion of results and related conclusions. Possible obstacles and potential solutions to the implementation of the project, and possible future research were also proposed.

2. Literature Review

The Powder River Basin has the largest coal resources in the United States (Bartos & Ogle, 2002). The coal seams contain trapped methane gas that was formed due to biogenic or thermogenic activity. To recover the methane in CBM reservoirs, the hydrostatic pressure that caused the adsorption of methane to the coal bed is reduced through the removal of water from the reservoir via CBM wells (McBeth *et al.* 2003) as simplified in Figure 2. The reduction in hydrostatic pressure initiates CBM gas desorption from the coal seams (Wheaton & Olson, 2001). Mostly, the volume of the CBM-produced water is massive at the beginning of production. However, as the amount of water in the coal decreases, the amount of methane production increases.

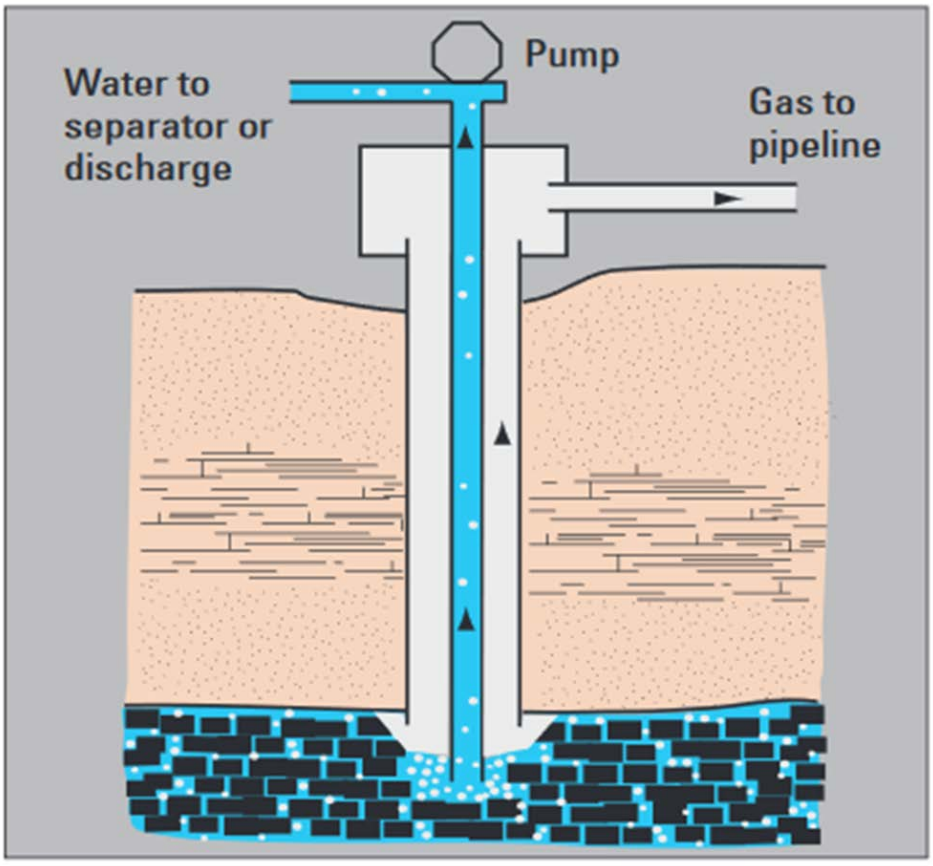

Figure 2: Simplified illustration of a CBM production well (USGS, 2000)

The consequence of pumping massive volumes of produced water includes lowering of the water levels in aquifers, soil erosion, surface water contamination, and disruption of natural water flows (Stearns *et al.* 2005). Owing to its direct contact with coal seams, most produced water has been known to contain many chemical constituents, as given in Table II, can limit its use. Typically, CBM produced water is characterized by elevated levels of salinity, sodicity, sodium, boron, barium, bicarbonates, manganese, and iron (ALL, 2003). These characteristics are of great threat to aquatic life when discharged into surface water or to crops when used for irrigation (Georgie *et al.* 2001).

Salinity is a measure of the content of total dissolved salts (TDS) in soil or water and is often measured by the movement of electricity through the water - electrical conductivity (EC). Generally, water with high TDS will conduct electricity better than a low TDS water. High concentrations of salt pose hazards to the environment as well as to the agriculture and infrastructures having wider economic impact. High levels of salinity in water and soil may cause plants to decline in biodiversity through dominance of salt-resistant species, potentially altering ecosystem structures ("salinity and water quality," 2012). The salinity level in the Powder and Little Powder Rivers and Mizpah Creek was given as 2,000 μS/cm by Horpestad *et al.* (2001).

Table II: CBM Produced Water Characteristics in the PRB (Veil et al. 2004)

Constituents	Minimum (mg/L)	Maximum (mg/L)	Mean (mg/L)
TDS	270	2010	862
SAR	5.7	32	11.7
Sodium	110	800	305
Calcium	5.9	200	36
Magnesium	1.6	46	16
Iron	0.02	15.4	0.8
Barium	0.1	8	0.6
Chloride	3	119	13
Sulfate	0.01	17	2.4

Sodicity in soil is the presence of a high proportion of sodium ions relative to other cations (Jackson & Reddy, 2007; Healy et al., 2011). When sodium makes up more than about 5% of all cations bound to clay particles, structural problems begin to occur in the soil. The sodium adsorption ratio (SAR) is the standard measure of sodicity. It relates the concentration of sodium to the sum of the concentrations of calcium and magnesium (see equation below):

$$SAR = \frac{Na^{+}}{\sqrt{\frac{1}{2}(Ca^{2+} + Mg^{2+})}}$$

where all concentrations are in meq/L.

A high SAR usually leads to reduced soil permeability which results to reduced infiltration and hydraulic conductivity culminating in surface crusting (Veil *et al.* 2004). Irrigation waters with SAR levels above 10 are considered sodic (Johnston *et al.* 2008). The SAR is lower in the southeast portion of the basin and increases towards the northwest.

2.1. CBM produced water discharge

Produced water management represents one of the most important operational considerations in CBM development. CBM production creates significant volumes of produced water with millions of gallons per day in many basins. Data from the Wyoming Oil and Gas Conservation Commission (WGOCC) shows a total of 5.4 billion gallons of produced water was produced in the Wyoming part of the PRB in 2016. Naturally, applicable legal and regulatory issues, produced water quality, the local environment and climate, and cost determine whether it should be managed as a waste product or put to beneficial use (ALL, 2003). Several water management options have been identified and some are currently being deployed for effective and beneficial use of CBM produced water. The water management options include well injection, impoundments, agricultural application, and membrane processes for domestic, municipal, and industrial use. According to the National Research Council (2010), almost 85 percent of all CBM produced water in the PRB is disposed of either by storage in surface impoundments or permitted direct discharge into ephemeral drainages and perennial rivers.

Due to its potential threat to the ecosystem, produced water discharge is under regulations in most states in the US. Montana exercises its authority to control or close river basin and groundwater aquifers to certain types of water appropriations because of water availability problems, water contamination problems, and a concern for protecting existing water rights. Accordingly, the PRB is one of the nine designated controlled areas for groundwater in Montana.

The control area applies only to wells designed and installed for the extraction of CBM (ALL, 2003).

The produce water disposal rule established by the Montana Board of Oil and Gas (MBOGC) stipulates guidelines based on its quality. Water with 15,000 ppm or less TDS can be retained and disposed of in a lawful manner that does not degrade surface waters, groundwater, or cause harm to soils while those with a TDS of more than 15,000 ppm should be disposed by Class II injection, into board-approved earthen pits at a monthly rate of less than 5 barrels per day, or can be temporarily stored in storage tanks or board-approved pits prior to injection. The board requires all discharges of produced water to comply with all applicable local, state, and federal water quality laws and regulations (ALL, 2003).

Currently, about 64% of produced water in the PRB are stored in lined impoundments designed for evaporative loss. Evaporative ponds are generally designed to be broad shallow pools that maximize the surface area allowing for high evaporation rates. Additional consideration is given to exposure to wind; areas with high winds and few natural (including low levels of vegetation) provide a relatively high evaporative potential (ALL, 2003). Table III shows the capacity and current volume of water being stored in CBM ponds operated by Summit Gas Resources, Inc.

Table III: CBM ponds operated by Summit Gas Resources, Inc.

Pond Name	Volume (Ac-ft)	Surface Area (Ac)
Big Nose Kate	194.38	13.40
Wild Bill Hickock pit #2	97.00	3.88
Sundance Kid	59.90	3.56
Porter 4-15	89.93	8.00
Doc Holiday pit #10	36.44	2.79
Rancholme 4-34 (Doc Holiday 2)	29.20	2.90
John Wayne	100.07	6.23
Calamity Jane #15	42.38	3.08
Jesse James	38.26	4.21
Bronco Billy	58.92	4.04
Total	**746.48**	**52.09**

2.2. Microalgae

Microalgae are microscopic photosynthetic unicellular organisms that are found in both marine and freshwater environments. The photosynthetic mechanisms of microalgae are similar to that of land plants, but microalgae are able to capture nutrients very efficiently out of their aquatic environment (Vandamme, 2013). Even with photosynthetic capabilities similar to that of higher plants, microalgae have a higher growth rate and productivity due to its smaller size and higher surface area (Mata *et al.* 2010). Under suitable growth conditions (light, nutrients, and temperature), microalgae can double their biomass within 24 hr (or 3.5 hr of exponential growth) (Chisti, 2007). There are a great variety of microalgal strains and they differ in their chemical and physical properties. Microalgal cells contain protein, lipids, polysaccharides, pigments and inorganic elements such as Cu, Fe, Se, Mn, and Zn. Interest in microalgal production has greatly

increased due to derivation of nutritional and other high-value products from its biomass. Algal products are commonly used for animal feed, biofuel, biofertilizers, chemicals, and high-value specialty products, as well as wastewater treatment (Slocombe & Benemann, 2016).

Some algal growths were observed at the CBM produced water ponds in the PRB of northeastern Wyoming and southern Montana (44° 52.613′N 106° 54.700′W). On October 18, 2010, a green alga - *PW95* was isolated at the produced water inflow into the pond shown in Figure 3. Partial DNA sequence analyses (18S and 16S) signified a unicellular culture, with no detected bacterial sequences, belonging to the *Chlorococcaceae* family (Hodgskiss *et al.* 2016) and sequencing revealed that it is in the genus *Neospongiococcum*.

Figure 3: CBM pond from where PW95 was isolated (Hodgskiss *et al.* 2016)

2.2.1. Microalgae as Biofertilizer

Organic fertilizer, hereinafter referred to as biofertilizer, are environmentally friendly, cost-effective, and sustainable alternatives to synthetic fertilizers. The use of algae as biofertilizer can be traced backed to the common use of seaweeds by ancient European and Asian farmers (Thirumaran *et al.* 2009). Their application are known to increase soil's nutrients and water-binding capacity, enhance production of antibiotics and enhance biodegradation of organic matter in the soil leading to necessary for enhancing crop yield (Critchley & Ohno 1998; Bhardwaj *et al.* 2014). These capabilities are due to the presence of various components such as plant growth hormones, regulators and promoters. Examples of such phytohormones are gibberellins, auxin, and cytokinin (Tarakhovskaya *et al.* 2007). *Cyanobacteria (Anabaena Cylindrica)*, a nitrogen fixing blue-green microalgae, have been widely used as a natural biofertilizer for rice production in countries such as India and Chile (Jha & Prasad, 2006; Pereira *et al.* 2009; Saadatnia & Riahi, 2009; Sharma *et al.* 2011). Likewise, application of *Chlorella vulgaris*, a green alga, stimulated and promoted growth of grape seedlings (Nanda *et al.* 1991).

2.2.2. Harvesting of Microalgae

Harvesting refers to the concentration of dilute microalgal suspensions, usually 0.02%–0.1% (200 mg/L – 1000 mg/L) total suspended solids (TSS) concentrated into 2%–25% TSS (Figure 4) or more depending on the end-product use (Davis, 2011). The concentration process has been estimated to account for up to 20-30% of the total production cost for microalgae (Grima *et al.* 2003; Zittelli *et al.* 2006).

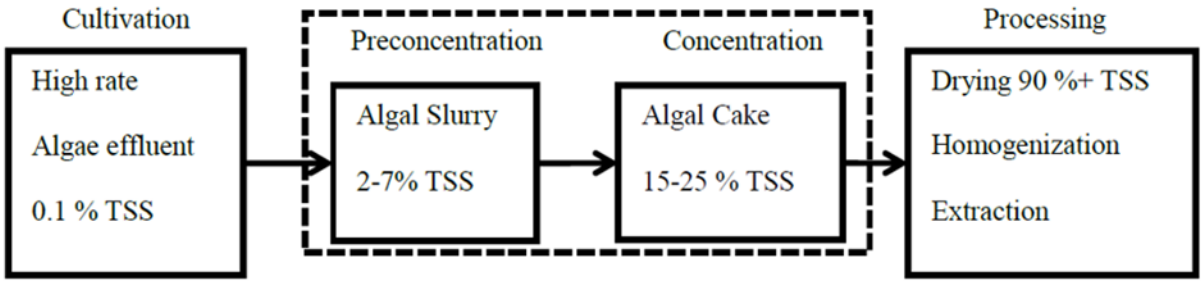

Figure 4: Block diagram of algal growth, harvesting, and processing (Davis, 2011)

The major harvesting techniques currently in use utilize common solid-liquid methods such as mechanical, electrical, and chemical separations (Christenson & Sims, 2011). Mechanical separations include centrifugation, filtration, sedimentation, and floatation techniques; electrical techniques utilize the electrophoresis of the microalgal cells; and chemical methods involve the use of flocculation techniques to concentrate algal cells (Liu *et al.* 2013). Despite these numerous separation techniques, harvesting algae from suspensions has proven to be one of the major obstacles in accessing its numerous benefits (Christenson & Sims, 2011; Weschler *et al.* 2014). The challenge in harvesting microalgae arises because of their density which is similar to that of water, small cell size (5~50 μm), negative surface charge (about −7.5~−40 mV), and the low biomass concentration of microalgal cells (Garzon-Sanabria *et al.* 2012; Milledge & Heaven. 2013). Therefore, to make microalgal biomass commercially viable, it is critical to develop a sustainable and effective harvesting process that is highly reliable with low capital and operational costs when applied at large scale. An optimum concentrating technique is species specific, depending on the properties of the culture medium and the quality of the product (Olaizola, 2003; González-Fernández & Ballesteros, 2013). The advantages and disadvantages of common separation techniques are enumerated in Table IV.

Table IV: Common microalgal harvesting techniques (Milledge & Heaven, 2013)

	Advantages	Disadvantages	Dry solids output conc' (%)
Centrifugation	Can handle most algal types with rapid efficient cell harvesting	High capital and operational costs	10–22
Filtration	Wide variety of filter and membrane types available	Highly dependent on algal species; best suited to large algal cells. Clogging or fouling an issue	2–27
Ultrafiltration	Can handle delicate cells	High capital and operational costs	1.5–4
Sedimentation	Low cost, potential for use as a first stage to reduce energy input and cost of subsequent stages	Algal species specific, best suited to dense non-motile cells. Separation can be slow. Low final concentration	0.5–3
Chemical flocculation	Wide range of flocculants available, price varies although can be low cost	Removal of flocculants, chemical contamination	3–8
Flotation	Can be more rapid than sedimentation. Possibility to combine with gaseous transfer	Algal species specific. High capital and operational cost	7

Unfortunately, microalgal characteristics have made it difficult to solely utilize only one of the presently available microalgal harvesting techniques (Vlaški *et al.* 1997). Hence, a two-stage harvesting process combining these techniques has been proposed. The first stage involves the bulk harvesting of the biomass from the microalgal suspension. Bulk harvesting refers to the first batch of cells taken from the microalgal suspension. Concentration factors for this operation are generally about 2% – 7% TSS. This efficiency is dependent on the initial biomass concentration and separation technologies employed. The second stage involves thickening of the slurry from bulk harvesting through separation techniques such as centrifugation, gravity separation, or filtration (Brennan & Owende, 2010).

Centrifugation of microalgae has proven to be very effective in harvesting almost all types of microalgal cells (Mohn, 1988). The gravitational force causes microalgal particles to separate from water due to a difference in densities. However, the process can be extremely slow due to microalgae's small particle size, and the small difference in density of microalgae and water (Milledge & Heaven, 2013). Furthermore, the process involves a high capital cost, and high operating cost (Moheimani *et al.* 2013). Similarly, exposure of microalgal cells, such as *A.*

cylindrica, to high gravitational and shear forces could damage cell structure (Knuckey *et al.* 2006).

Gravity sedimentation provides the least costly and simplest method of concentrating microalgal biomass (Shelef *et al.* 1984; Uduman *et al.* 2010). Another harvesting technique is floatation of microalgal cells. This process involves bubbling air or gas through dilute microalgal suspension with the gaseous molecules attaching to the microalgal cells. These algal particles move to and aggregate on the liquid surface where they are removed. Generally, the floatation harvesting technique can be relatively faster and more effective than the sedimentation technique (Chen *et al.* 1998; Chung *et al.* 2000).

Filtration is another option to concentrate microalgal cells (Frappart *et al.* 2011). This process separates suspended microalgal cells from its suspension by the passage of the suspension through a porous medium usually with a pore size of 0.1 microns and above (Crittenden *et al.* 2012). Liquid effluents from the permeable medium will have little or no microalgal cells. However, the performance of this process is usually affected by low throughput and rapid clogging of the membrane (Mohn, 1988; Oswald, 1988). Flocculation of algal cells has been touted to be an efficient harvesting process that has found application in the industries. The process occurs when the suspended algal cells in the suspension form an aggregate commonly referred to as flocs.

2.2.2.1. Flocculation

Flocculation is a process in which dispersed microalgal cells aggregate and form larger particles with higher sedimentation rate. The process has been determined to be a superior method for harvesting algae when compared to other methods because of its effectiveness in concentrating large quantities of microalgal culture and a wide range of algal species (Pushparaj

et al. 1993; Lee *et al.* 1998). Investigations of several harvesting methods by Schenk *et al.* (2008) stated that flocculation combined with flotation or sedimentation and subsequent further dewatering by centrifugation or filtration is the most promising cost and energy efficient alternative. Benemann *et al.* (1980) also described the process as the most reliable and relatively cost-effective method of concentrating algae. When utilized as a bulk harvesting process, the process alone can achieve a concentration factor of 100 – 800 times to reach 2 – 7% TSS (Brennan & Owende, 2010). Formation of flocs can be individually or collectively attributed to mechanisms such as charge neutralization, electrostatic patch mechanism, polymeric bridging, and sweep flocculation (Vandamme *et al.* 2013).

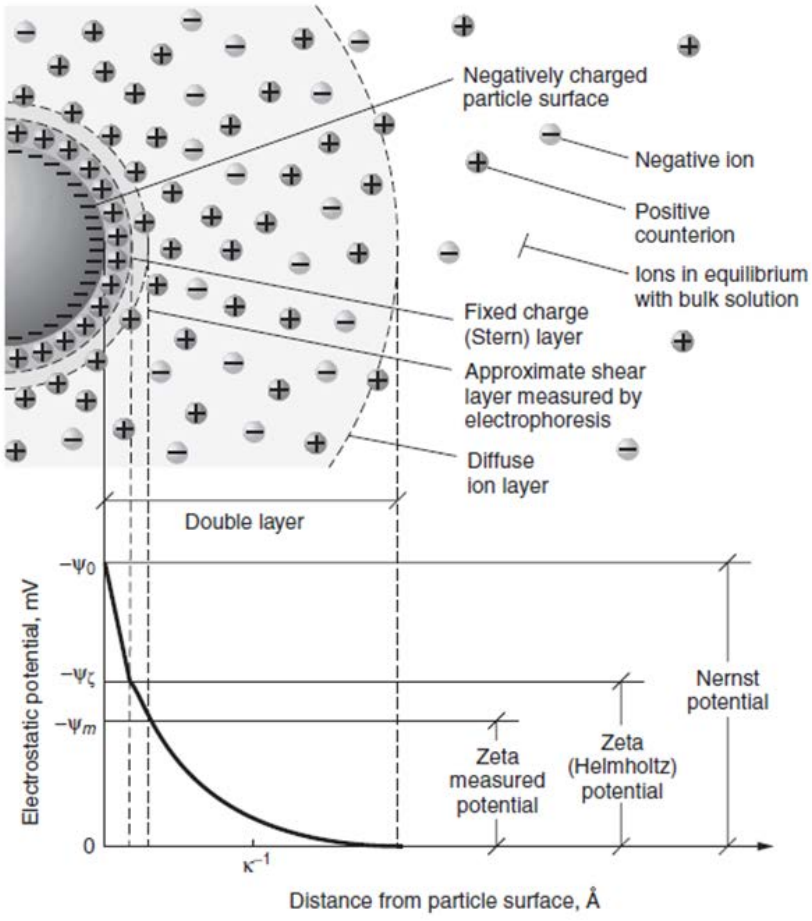

Figure 5: Structure of the electrical double layer (Crittenden *et al.* 2012)

The electrical double layer (EDL) shown in Figure 5 can be used to explain the stability of microalgal cells in an aqueous suspension. The negative surface charge, originating from the presence of water-soluble amino and carboxyl groups, attracts positively charged ions from the surrounding aqueous to form a fixed adsorption layer of cations of about 5˚A thick and is known as the Helmholtz or Stern layer (Crittenden *et al.* 2012). Beyond this layer is the diffused layer consisting of excess cations repelling anions and extending deep into the bulk solution until electroneutrality is achieved. Kruyt *et al.* (1952) stated that the EDL (combination of the Stern and diffuse layers) can extend up to 300˚A into the solution. The measure of the electrical potential between the actual shear plane in the diffuse layer and the bulk solution is given by the zeta potential. The zeta potential is a measure of the electrostatic potential at the algal cell's surface boundary, and it is directly proportional to the strength of the algal surface charge per unit area (Reynolds, 1982). Hence, a large zeta potential signifies a large repulsion forces between the microalgal cells translating a stable algal suspension. For cell aggregation to occur, the flocculant used must overcome the formed energy barrier by effecting an increase in the ionic strength of the solution. An increase in ionic strength leads to a reduction in the size of the EDL, and the zeta potential between the microalga cells. Rapid flocculation, brought about by van der Waals force, will occur when the EDL is less than 10 Å and the zeta potential is approximately 20 mV (Crittenden *et al.* 2012).

Flocculation can occur spontaneously in some microalgae (autoflocculation), be induced by the addition of flocculants, and can be stimulated (bioflocculation). Autoflocculation refers to spontaneous aggregation of microalgal cells without the influence of added chemical flocculants (Ayoub *et al.* 1986; Brady *et al.* 2014). The process occurs in response to changes in culture conditions such as nitrogen limitation, light, pH and dissolved oxygen (Schenk *et al.* 2008;

Uduman *et al.* 2010). Overlying separation mechanism is associated with an increase in culture pH level brought about by CO_2 consumption by the algae during photosynthesis and the precipitation of inorganic precipitates (Sukenik & Shelef, 1984; Uduman *et al.* 2010). The presence of excess ions in phosphate-rich medium explains why autoflocculation does not happen in all microalgal suspensions. A major disadvantage of autoflocculation is that it is unreliable, uncontrollable, slow, and may induce undesired changes in cell composition (Benemann and Oswald, 1996; Schenk *et al.* 2008).

Bioflocculation is a cost and energy efficient alternative for concentrating microalgae using a naturally flocculating microalga to concentrate the non-flocculating microalgae of interest. It is assumed to be caused by dissolved extracellular polymeric substances (EPS), transparent extracellular particulate (TEP) in the medium (Larkum *et al.* 2012; Manheim & Nelson 2013), and aggregation between microalgae and bacteria (Kogure *et al.* 1981). Both EPS and TEP have been described as sticky substances with TEP being larger. Passow *et al.* (2001) describes TEP as a substance formed from the dissolved organic matter excreted naturally from algae. The spontaneous flocculation is believed to be brought about by the bridging capabilities of the algae-excreted EPS (Pavoni *et al.* 1974). Due to repulsion caused by the negatively-charged cell membranes, not all microalgal species can bioflocculate. However, bioflocculating algal species can be used in inducing flocculation of other non-flocculating species (Schenk *et al.* 2008; Taylor *et al.* 2012). Bioflocculation has shown to be successful with bacteria (Lee *et al.* 2009) and fungi (Zhou *et al.* 2012). However, it demands an additional substrate and energy source for bacterial or fungal growth, which will evoke undesirable bacterial or fungal contamination of the microalgal production plant.

Flocculation can also be achieved using flocculants to induce cell aggregation. The addition of flocculating agents reduces or shields the negative charge of the microalgal cells (McGarry, 1970; Lee at al. 1998; Papazi *et al.* 2010). Flocculation can be induced through a decrease or an increase in medium's pH with the addition of an acid or a base respectively. According to Liu *et al.* (2013), the use of an acidic flocculant may change carboxylate ions into neutral carboxyl groups leading to charge neutralization, cell agglomeration, and settling by gravity. Acid-induced flocculation can be observed at a pH of 5 and it is optimum between 4 to 2 (Liu *et al.* 2013). In view of large volume of culture required for processing, the use of acid flocculants is not economical for low-value products like biofuel (Thiruvarasn *et al.* 2015). Unlike the flocculation induced by acid, flocculation induced by pH increase for harvesting microalgae can allow for the reuse of flocculated medium. Depending on cell density (Schlesinger *et al.* 2012; Besson & Guiraud, 2013), induced flocculation via pH increase has been observed at a pH of 9 -12 (Blanchemain & Grizeau, 1999; Ras *et al.* 2011; Spilling *et al.* 2011; Huo *et al.* 2014).

Commonly used flocculants include metallic salts (alum and iron sulfate), polyelectrolytes, and alkaline compounds (NaOH, KOH, $Ca(OH)_2$, $Mg(OH)_2$) (Lee *et al.* 1998; Papazi *et al.* 2010). The use of metallic salts has proven to be the most efficient technique of achieving flocculation (achieving over 90% efficiency). Aluminum sulfate is preferred to ferric sulfate because of lower optimal dosage, pH and the quality of the resultant effluent and product (Sukenik & Shelef, 1984). The use of these metal salts is not attractive in terms of environmental pollution, operational cost and the quality of the end products arising from biomass contamination (Chen *et al.* 2011).

Polyelectrolyte flocculants are basically polymer flocculants consisting of ionic and non-ionic species, natural and synthetic polymers. Flocculation is achieved through a combination of charge neutralization and bridging of the polymeric molecule to the surface of the microalgae via electrostatic or chemical forces, extent of charge density and polymer chain length (Uduman *et al.* 2010). The extent of polymer-algal molecule adsorption and bridging is greatly determined by the molecular weight of the cationic polymer deployed. Since algal surfaces are anionic in natural waters (Brady *et al.* 2014), a polymer flocculant needs to be positively charged. Hence, a cationic biopolymer -Chitosan- is widely used as a flocculant and it is very efficient at low pH (Chang & Lee, 2012). Table V shows a comparison of these different flocculants.

Table V: Comparison of chemical flocculants for algal harvesting (Wan *et al.* 2015)

Chemical flocculants (Dosage)	Classification	Microalgae (cell density)	FE[a] (settle time)	Features	References
$Al_2(SO_4)_3$ (0.1 g L^{-1}) $FeCl_3$ (0.2 g L^{-1})	Inorganic	*C. zofingiensis* (0.5 g L^{-1}) *Scenedesmus* sp. (0.23 g L^{-1})	>90% (60 min)	pH depended, and risk of secondary pollution	Chen et al. (2013) Wyatt et al. (2012)
$Fe_2(SO_4)_3$ (1 g L^{-1})	Inorganic	*C. minutissima* (2.2 × 108 mL^{-1})	>99% (60 min)	High dosage, cell damages, and risk of secondary pollution	Papazi et al. (2009)
Aluminum nitrate sulfate (5.4 mg L^{-1})	Inorganic	*N. salina* (10–20 g L^{-1})	>95% (30 min)	Residual aluminum in mciroalgal biomass	Rwehumbiza et al. (2012)
Ammonia (38–120 mM)	Inorganic	*C. sorokiniana* (–[b]) *Dunaliella* sp. (–) *N. oculata* (–)	>85% (180 min)	Reuse of medium, long flocculating time, and species dependent	Chen et al. (2012)
Polyacrylamide (0.05 g L^{-1})	Inorganic polymer	*Scenedesmus* sp. (0.54 g L^{-1})	60% (10 min)	High pH depended, and risk of toxic acrylamide	Chen et al. (2013)
Polyaluminum chloride (20–40 mg L^{-1})	Inorganic polymer	*N. gaditana* (132 mg L^{-1}) *P. tricornutum* (105 mg L^{-1})	70–80% (30 min)	pH sensitive, and risk of secondary pollution	Şirin et al. (2013) Şirin et al. (2011)
Polyelectrolyte EM1 (15 mg L^{-1})	Inorganic polymer	*Muriellopsis* sp. (2 g L^{-1})	90% (15 min)	Risk of secondary pollution	Granados et al. (2012)
Chitosan (20–30 mg L^{-1})	Organic polymer	*C. sorokiniana* (2 g L^{-1}) *N. gaditana* (132 mg L^{-1}) *P. tricornutum* (105 mg L^{-1}) *Scenedesmus* sp. (0.23 g L^{-1})	60–99% (30 min)	Favor pH depended, and high cost of chitosan	Xu et al. (2013) Şirin et al. (2013) Şirin et al. (2011) Chen et al. (2013)
Nano-chitosan (60 mg L^{-1})	Organic polymer	*Nannochloropsis* sp. (665 × 106/mL)	96% (60 min)	Reuse of medium, and high cost of chitosan	Farid et al. (2013)
Cationic starch (20–40 mg L^{-1})	Organic polymer	*C. prototheeoides* (0.56–0.77 g L^{-1})	79–90% (60 min)	pH depended	Leqtelier-Gordo et al. (2014)
Moringa oleifera seed flour (1 g L^{-1})	Organic polymer	*C. vulgaris* (OD_{730} 0.9–1.06)	88% (120 min)	High dosage, and alkaline pH favored	Teixeira et al. (2012)
Poly γ-glutamic acid (γ-PGA) (~20 mg/L)	Organic polymer	*C. prototheeoides* (0.6 g L^{-1}) *N. oculata* (0.6 g L^{-1}) *P. tricornutum* (0.6 g L^{-1})	>90% (120 min)	Salinity depended, long flocculating time	Zheng et al. (2012)

[a] FE – flocculation efficiency.
[b] Data of concentration is unavailable.

High flocculation efficiency has also been observed when an algal suspension is flocculated using alkaline compounds such as NaOH, KOH, $Ca(OH)_2$, $Mg(OH)_2$. Cell aggregation mechanism is predominantly caused by charge neutralization of the algal surface charge (Sangeetha *et al.* 2015). Numerous studies have indicated that sodium hydroxide (NaOH) showed a better flocculation capability at lower volume than potassium hydroxide (KOH). With intended use of resulting biomass as biofertilizer, sodium remnants in the biomass would be toxic for plant life unlike potassium which is known to sustain plant growth and reproduction.

3. Materials and Methods

The fertilization effects of microalgae were evaluated using two economically important crops of Montana: Winter wheat (*Triticum aestivum*), Ranger Russet seed potato (*Solanum tuberosum*), and Flax (*Linum usitatissimum*). *PW95* isolates from the CBM ponds (sequenced as *Neospongiococcum sp.*) and *Anabaena cylindrica* cells were obtained from the Center for Biofilm Engineering at Montana State University. For the fertilization experiments, two brands of chemical fertilizers, Stern's Miracle-Gro – THERAPY Plant Food (10-8-7) and Hoagland Solution, were used. Both brands were prepared for application at the label directed rates. The soil used for all the fertilization experiments was a Sunshine Professional Growing Mix .1-.1-.1 (Product #:0103-0010) manufactured by the Sun Gro Horticulture Canada Ltd (Appendix H).

3.1. Cultivation of Microalgae

The microalgae were cultured in Erlenmeyer flasks (0.5 – 4.0L) each capped with a rubber stopper. To avoid contamination, all autoclavable materials were sterilized in an autoclave. As shown in Figure 6, the microalgae were grown in freshwater with controlled nutrients, lights, and temperature.

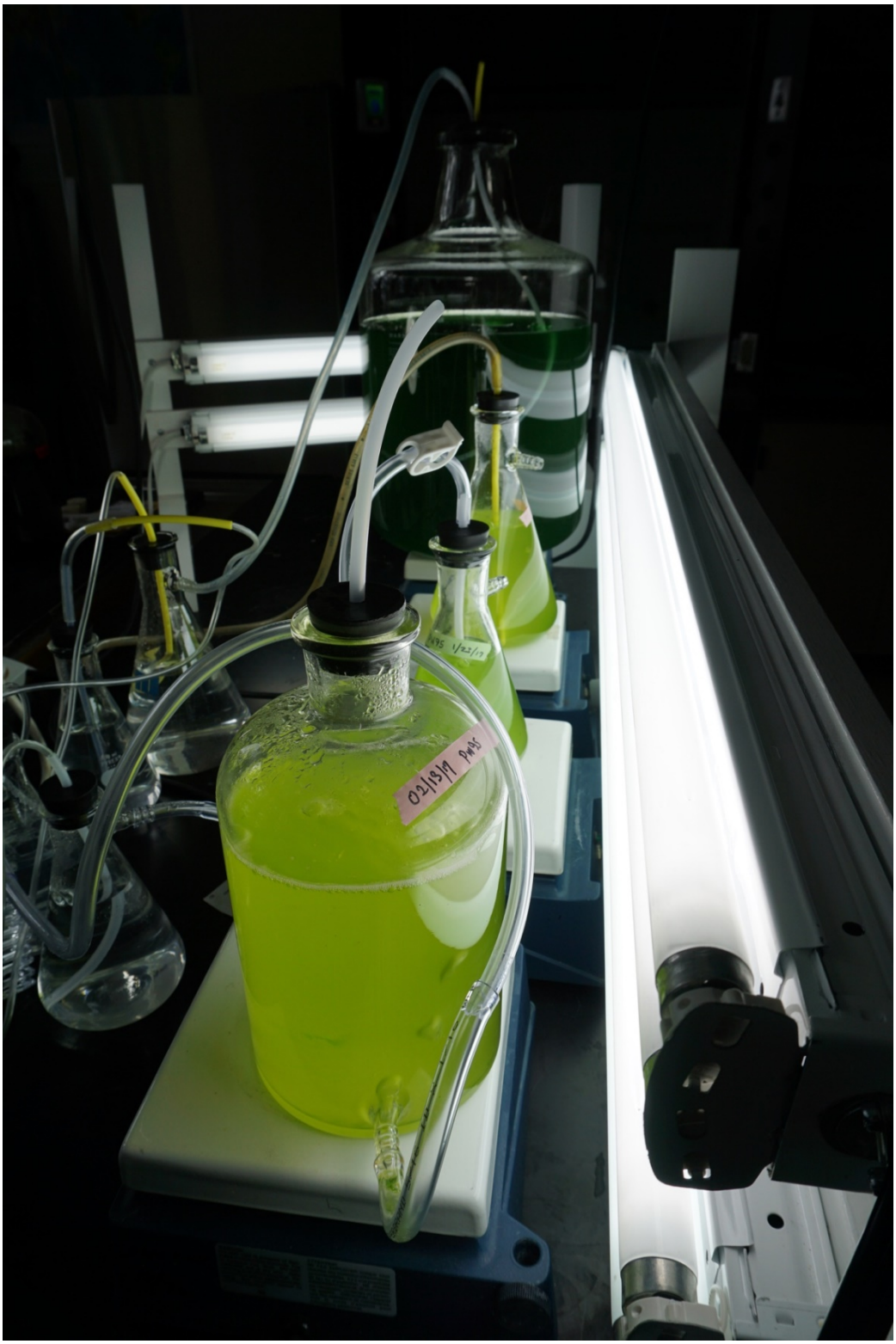

Figure 6: PW95 and A. cylindrica Culturing set-up

PW95 was cultured with B1650 Bold's Basal Medium (BBM), with a KOH adjusted pH of 6.6 +/- 0.1. The BBM is a predominantly inorganic freshwater medium used for culturing green algae and does not require additional soil extracts or vitamins (Brown *et al.* 1964; Nichols and Bold, 1965). BG-11 (Blue-Green) Medium Solution (50x), another freshwater medium, was used to culture *A. cylindrica*. Both sterile-filtered solutions were obtained from Phyto-Tech, Inc. Tables VI and VII show the chemical constituents, as provided by Phyto-Tech, Inc., of the BBM and BG-11 media respectively.

Table VI: Chemical constituents of BBM (50x)

Constituents	Concentrations (mg/L)
Boric Acid	571
Sodium Nitrate	12,500
Manganese Chloride.$4H_2O$	72
Calcium Chloride, Anhydrous	943.6
Sodium Molybdate	59.7
Cobalt Nitrate.$6H_2O$	24.5
Cupric Sulfate.$5H_2O$	78.5
Potassium Phosphate - dibasic	3,750
Potassium Phosphate – monobasic	8,750
EDTA, Disodium Salt	3,180.5
Ferrous Sulfate.$7H_2O$	249
Sodium Chloride	1,250
Zinc Sulfate.$7H_2O$	441
Potassium Hydroxide	1,550
Magnesium Sulfate, Anhydrous	1,831.3

Table VII: Chemical constituents of BG-11 (Blue-Green) Medium (50x)

Constituents	Concentrations (mg/L)
Boric Acid	143
Sodium Nitrate	75,000
Manganese Chloride.$4H_2O$	90.5
Calcium Chloride, Anhydrous	1,359
Sodium Molybdate.$2H_2O$	19.5
Cobalt Nitrate.$6H_2O$	2.45
Cupric Sulfate.$5H_2O$	3.95
Potassium Phosphate – dibasic	2,000
Ferric Ammonium Citrate	300
Na_2.Mg.EDTA	50
Magnesium Sulfate, Anhydrous	3,750
Sodium Carbonate, Anhydrous	1,000
Zinc Sulfate.$7H_2O$	11.1
Citric Acid, Anhydrous	300

Both cultures were prepared by adding 20 mL of the media per liter of distilled water, grown at 15 °C (59 °F), and illuminated on a 14h/10h light/ dark cycle using cool-white 32W 6500k fluorescent lights at an exposure of 11,000 Lux measured with a light intensity meter (Extech Instruments - L374679). The cultures were also bubbled with filtered and hydrated air to keep the algae in suspension so as to enhance effective gas exchange through mixing, and increase exposure to light. The hydrated air was delivered through flexible tubing by an air pump (Active Aqua AAPA15L).

3.1.1. Absorbance and Optical Density Measurements

Optical density is the log ratio of transmitted light to incident light while absorbance is the capacity of a substance to absorb light of specific wavelength. Generally, both parameters measure the amount of light that is "absorbed" when passing through an optical component.

Microalgal cell concentration was determined through the optical density of the culture and measured at 750 nm using a Thermo Spectronic Genesys™ 20 visible spectrophotometer wavelength range of 325 nm to 1100 nm and a ± 2.0 nm accuracy. The optical density was measured at 750 nm, rather than 680 nm, because measurements at the latter wavelength would limit inaccuracies that can be introduced when the pigment content of the cells changes (Griffiths *et al.* 2011). Deionized water was used as the reference sample and the cell samples were diluted and placed in a semi-micro polystyrene cuvette, BrandTech 759076D, with a path length of 10 mm.

3.1.2. Dry Weight Measurements

The dry weight of biomass per volume of the suspension of *PW95* was determined by filtering 5 to 20mL aliquot through a 0.45 µm nitrocellulose filter with a diameter of 47 mm. The used filter, with the suspended solids, was subsequently placed in an oven set at 104°C for 24 hours. The final dry weight represents the net weight difference of the filter before filtering and after drying. The dry weight of *PW95* biomass per volume was calculated as:

$$\frac{g}{mL} = \frac{net\ weight\ difference\ of\ filter\ before\ and\ after\ filtration\ and\ drying\ (g)}{filtration\ volume\ (mL)}$$

3.1.3. Estimating Cell Density

Cell density was measured by introducing 10µL of cell suspension to the v-shaped groove on each side of a hemacytometer. The number of cells in different corner squares of the hemacytometer were counted under a microscope. Cell density was calculated using the equation below:

$$Cells/mL = \frac{Average\ cell\ count\ x\ dilution\ factor}{Volume\ of\ a\ corner\ square\ (mL)}$$

Where the average cell count was obtained by dividing the total number of cells counted by the number of squares counted. The dilution factor represents the extent of dilution of the original concentration in order to enhance uniform cell distribution and avoid cell overlap that will result in counting error. The volume of each corner square is 0.1 mm3 (0.0001 mL) and is derived by multiplying the area of the corner square (1 mm^2) by the depth of the square (0.1 mm).

3.2. Biomass Harvesting

3.2.1. Gravitational Sedimentation

The gravitational harvesting method was used to concentrate the algal biomass by pouring the suspension into an Imhoff sedimentation cone. This method was chosen because it has the advantage of keeping intact the algal cell structure and producing a concentrate that is free of contaminants and chemical flocculant residue. However, the main disadvantage is the slow separation and low final concentration.

3.2.2. Induced Flocculation Process

Fifty mL of 0.1M KOH solution was prepared by dissolving 0.281g of KOH pellets in deionized water. An Accumet AB 15/15+ bench-top pH meter was used to measure the initial pH of the algal suspension. To enumerate cell concentration, an aliquot (3 mL) of the suspension was placed in a cuvette and was used to measure the absorbance of the suspension at 750 nm.

Fifty mL of the cell cultures was transferred into a 100 mL Griffin beaker placed on a "Fisher Scientific" magnetic stir plate and the pH of the suspension was gradually increased dropwise by the addition of 0.1 M KOH using a pipette set to a volume of 0.1 mL. Separate 50 mL cultures were used for each pH level (at intervals of one complete pH unit: 10 to 12). The cultures in the beaker were continuously mixed at 250 rpm throughout the experiment with the aid of a magnetic stir bar. During titration, an Accumet AB 15/15+ bench-top meter was used to

monitor the increasing pH steps. KOH titration was stopped once a desired pH step was attained. The mixing then continued for another 1 min and for an additional 2 minutes at 60 rpm to encourage floc formation. The whole suspension was then gently poured into a 100 mL graduated cylinder while slanting the graduated cylinder at an angle so as to reduce the shear impacted on the flocs as they were transferred to the graduated cylinder. The cultures were allowed to settle in the cylinder for a period of 15 minutes (Sangeetha *et al.* 2015; Thiruvarasn *et al.* 2015).

3.2.2.1. Measuring Optical Density during Flocculation

Optical density and absorbance values are crucial in determining the removal efficiency and final concentration factor of the culture. Hence the absorbance measurements were conducted by pipetting 3 mL aliquots of supernatant at two-thirds of cylinder height from the bottom of the graduated cylinder (Figure 14). The aliquot was taken at this point so as to prevent any re-suspension of the cells. The collected aliquot was subsequently submitted for absorbance measurement at 750 nm using the Thermo Spectronic Genesys™ 20 visible spectrophotometer.

3.2.2.2. Flocculation Efficiency

The flocculation efficiency (FE) can be used to characterize the biomass recovery using the formula below:

$$FE\ (\%) = \left(\frac{A - B}{A}\right) \times\ 100$$

where A is the optical density of the supernatant taken before KOH titration and B is the optical density of the supernatant after a specified time of settling.

3.3. Biomass requirement and application

After biomass harvesting, representative samples of *PW95* (4.6 g/L) were sent to the Soil, Plant, and Water Laboratory of the University of Georgia for chemical constituent analysis. The results are presented in Table VIII.

Table VIII: Nutrients composition of *PW95*

Constituents	Ave. Concentrations (mg/L)	S.D
Calcium	23	4.83
Potassium	263.89	43.71
Magnesium	42.15	15.41
Phosphorus	130.01	54.8
Nitrogen	1240	293.6
Sulphur	34.6	11.51
Aluminum	5.87	0
Boron	1.97	0.39
Cadmium	<0.4	<0.4
Chromium	<0.5	<0.5
Copper	2.22	2.38
Iron	6.79	5.52
Manganese	1.5	0.5
Molybdenum	<0.5	<0.5
Sodium	115.2	17.3
Nickel	<0.5	<0.5
Lead	<1.0	<1.0
Zinc	3.88	0.59

Nitrogen is usually the most important of all the plant nutrients. Hence, biomass application was applied per the nitrogen specification in chemical fertilizer requirement of the crop to be planted and the surface area of the pot. For example, winter wheat (*T. aestivum*) is very sensitive to nitrogen (N) insufficiency and requires about 22.42 kg N/ha (2,242 mg N/m^2).

The specific amount of biomass to be added is then determined by multiplying this value (2,242 mg N/m^2, 1.8 L/m^2) by the surface area of the greenhouse nursery pot.

Individual winter wheat seedlings were planted in each of 90 one-gallon greenhouse nursery pots filled with 3.25L of soil. The pot have five drain holes and dimensions of 15.88 cm and 16.51 cm for depth and diameter respectively. Subsequently, the pots' surface area was determined as 33.183 square inch (0.02141 m^2) leading to a biomass requirement of 48 mg N/pot. Of the total of 90 winter wheat seedlings planted, a chemical fertilizer (M.Gro) was added to 30 pots and algal biomass was applied to another 30, while the remaining 30 served as the control group receiving only water.

3.3.1. Pigment Content measurement

Chlorophyll content of crop leaves was determined by using a Chlorophyll Content Meter (CCM 300, Opti-Sciences, Inc.). The measured parameter is the Chlorophyll Fluorescence Ratio (CFR) - fluorescence emission ratio of intensity at 735 nm/700 nm, with a readout of relative chlorophyll content in mg/m^2 with a noise of <+/- 2%.

4. Results and Discussion

Data and observations from various investigations of biomass harvest through flocculation and evaluation of the fertilization properties of *PW95* and *A. cylindrica* biomass are presented in this section. The first part includes the data obtained from the fertilization of wheat, potato, and flax with *PW95* biomass harvested through the sedimentation by Imhoff cone. The difference in results from the fertilization were discussed. Results of the induced flocculation were presented in the second part of the section. Relevant parameters depicting the efficiency of the flocculation separation method were also discussed.

4.1. Fertilization Investigation

Parameters such as chlorophyll content, plant height and number of tillers (for wheat only), seedling weight were evaluated in the fertilization experiments for *T. aestivum, S. tuberosum,* and *L. usitatissimum*. Figure 7 shows the filtrate from the soil after the addition of *A. cylindrica* to a potato pot.

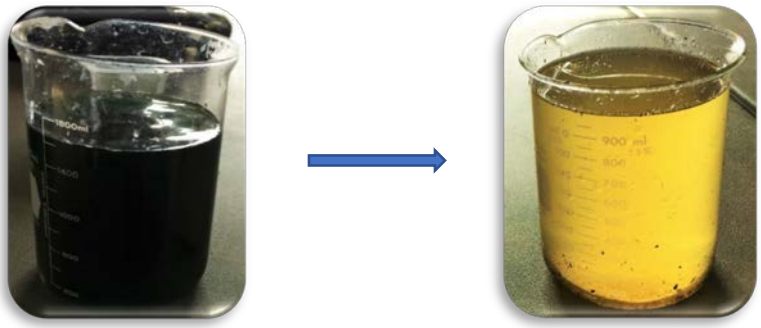

Figure 7: Filtrate from *A. cylindrica* application to soil

4.1.1. Wheat

Figure 8 shows the difference in plant height of wheat with three treatments. One-way analysis of variance shows a significant difference in average wheat height. *PW95*-fertilized wheat was significantly taller than the M.Gro and control group on day 7. This effect

subsequently disappeared on days 13, 33, and 67. This disappearance is believe to be connected to the fact the *PW95*-fertilized pots had more tillers (shown in Table IX). Overall, M.Gro-fertilized wheat showed a significantly taller wheat stem than those fertilized by *PW95* and water on days 33 and 67.

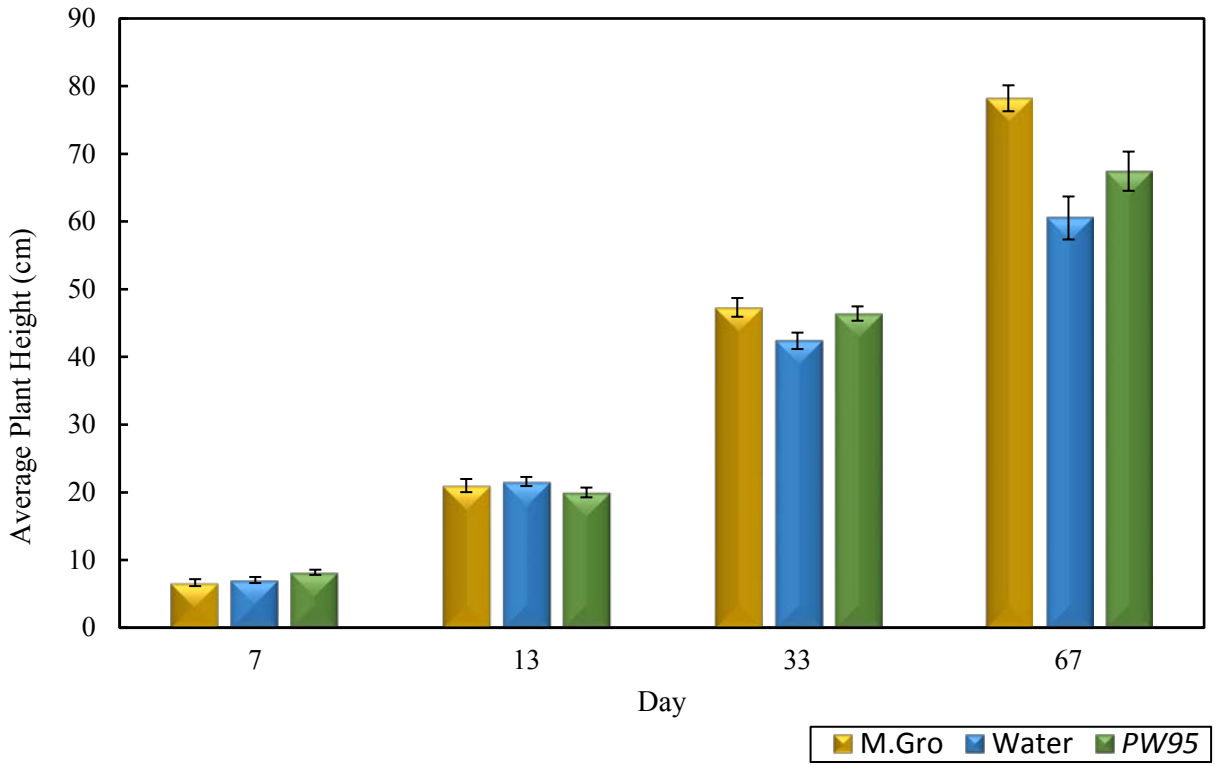

Figure 8: Wheat Height Plot

Chlorophyll is essential in photosynthesis, allowing plants to absorb energy from light. It is an indirect measure of nitrogen content and an indicator of plant health. Evaluation of chlorophyll content of the plants indicated a consistent trend of chlorophyll reduction as the plants reach maturity (Figure 9). Pots treated with M.Gro and *PW95* both showed higher chlorophyll contents when measured at day 11 while the control pots showed lower chlorophyll content. The sudden drop in chlorophyll content is typical of wheat plants after it blooms and

make grains. Hence, the chlorophyll measurement on days 33 and 67 were done on another leave in the pot.

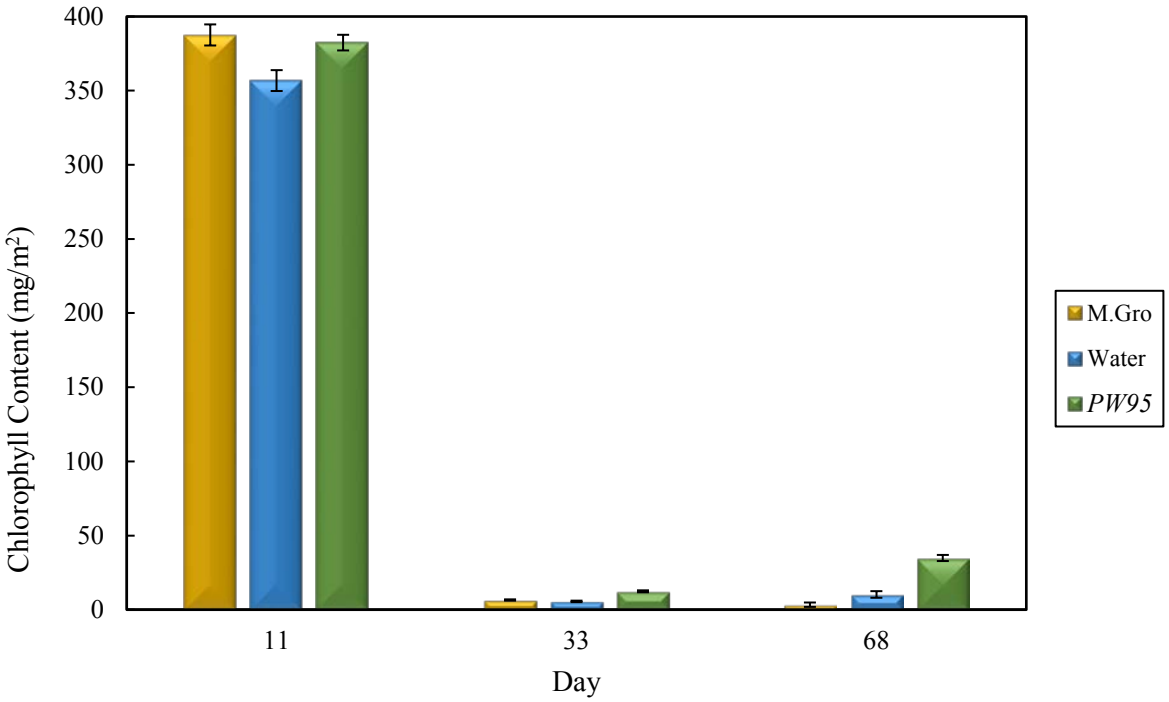

Figure 9: Wheat Chlorophyll Content

On day 33, measurements of some other parameters (such as number of pots with brown leaves, pots with tillers) showed that *PW95* – fertilized plants had more leaves and flowers, fewer brown leaves, and more tillers. Tiller refers to all shoots that grow after the initial parent shoot grows from a seed. Water-treated wheat posted similar results with M.Gro treated plants but they came far behind in number of tillers as shown in Table IX.

Table IX: Other measured growth parameters

	Treatments		
	M.Gro	**Water**	***PW95***
Ave. # of Leaves	6	6	8
Brown Leaves	30	30	9
Tillers	17	3	25
Flowers	2.73	2.53	3.47

Overall, *PW95*-treated wheat had the highest yield and average total seed weights when compared to M.Gro-, and water-treated wheats (Figure 10). This could be directly related to the data in Table IX showing that *PW95*-fertilized produced the highest number of seed-bearing flowers. Also, the average total dry weight for *PW95*-fertilized wheat was 117% and 47% more than those of water-, and M.Gro-treated wheats respectively. Likewise, measurements of the seed weights showed that weights of *PW95*-treated plants were 123% and 58% more than corresponding measurements for wheat treated with water and M.Gro respectively. Finally, *PW95*-fertilized wheats posted an average of 3.5 flowers which is 37% and 27% more than the average flowers produced by wheat fertilized by water and M.Gro respectively.

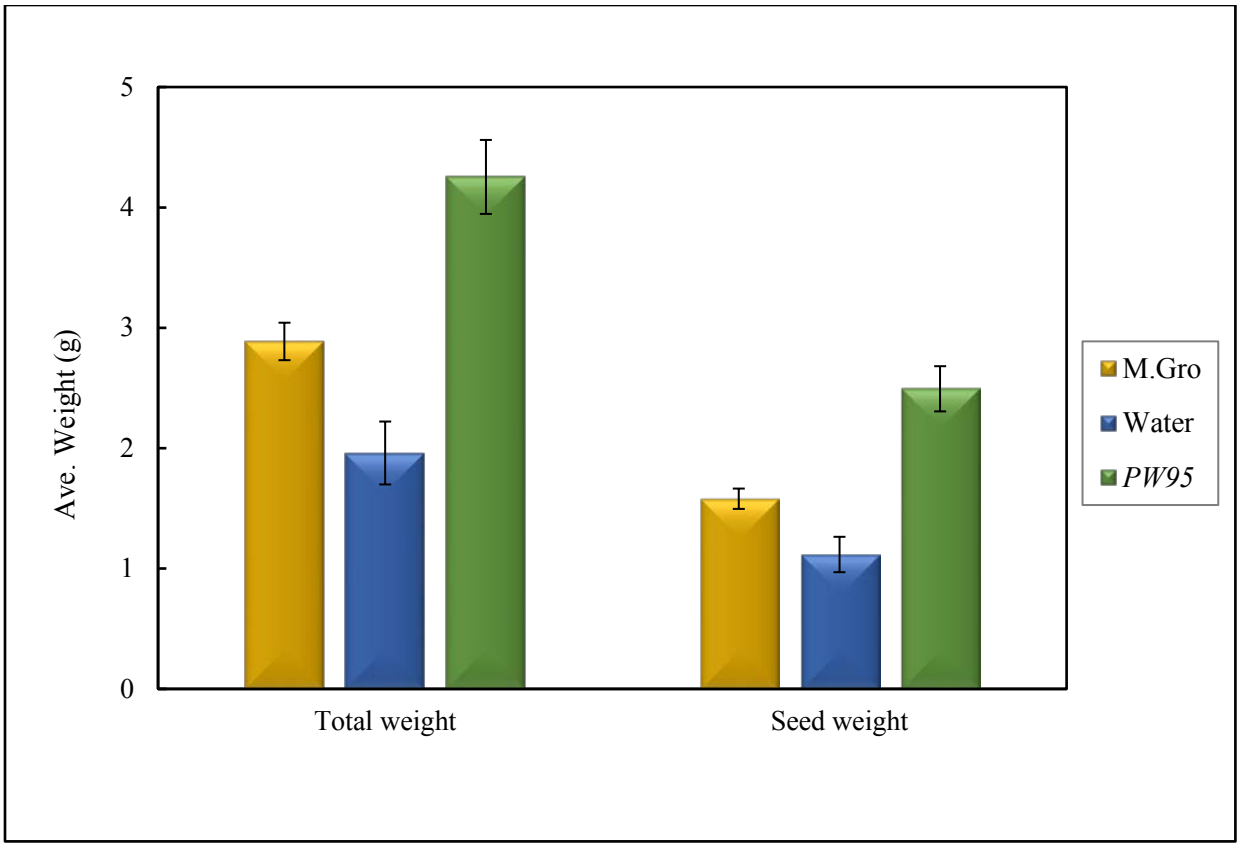

Figure 10: Wheat Yield Plot

4.1.2. Potato

Thirty (30) potato tuber-seedlings were planted in 5-gallons grow bags (dimension of 9" x 8" x 16.5") filled with 3L soil. The tuber-seedlings were fertilized with *PW95*, Hoagland solution (HS), and water. *PW95*-treated potatoes showed the highest chlorophyll content at an average of 232 mg/m^2 and 189 mg/m^2 at days 68 and 88 respectively. The control plant posted the lowest chlorophyll content of the three treatments with 198 mg/m^2 and 157 mg/m^2 at days 68 and 88 respectively (Figure 11).

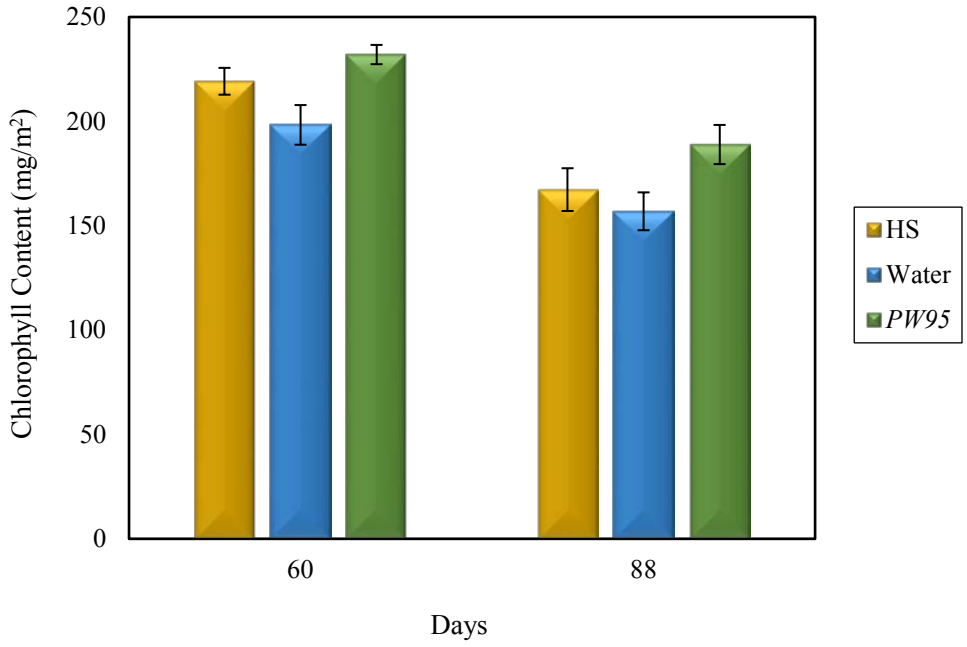

Figure 11: Potato Chlorophyll Content

Unlike microalgae-treated wheat, Figure 12 shows that potatoes treated with water had the best harvest when compared to potatoes fertilized with both *PW95* and chemical fertilizer. This result might probably be related to the higher chlorophyll content posted by plants with *PW95* and HS treatments in Figure 11. With chlorophyll content being a great indicator of nitrogen content in leaves, it is postulated that excess nitrogen in the potatoes fertilized with HS and *PW95* led to a reduced tuber formation. Generally, root crops do not require huge application of nitrogen as it only results in the development of a great crop of potato plants with poor tuber growth. Alternatively, it is possible that microalgae might only be suitable for monocots than dicots.

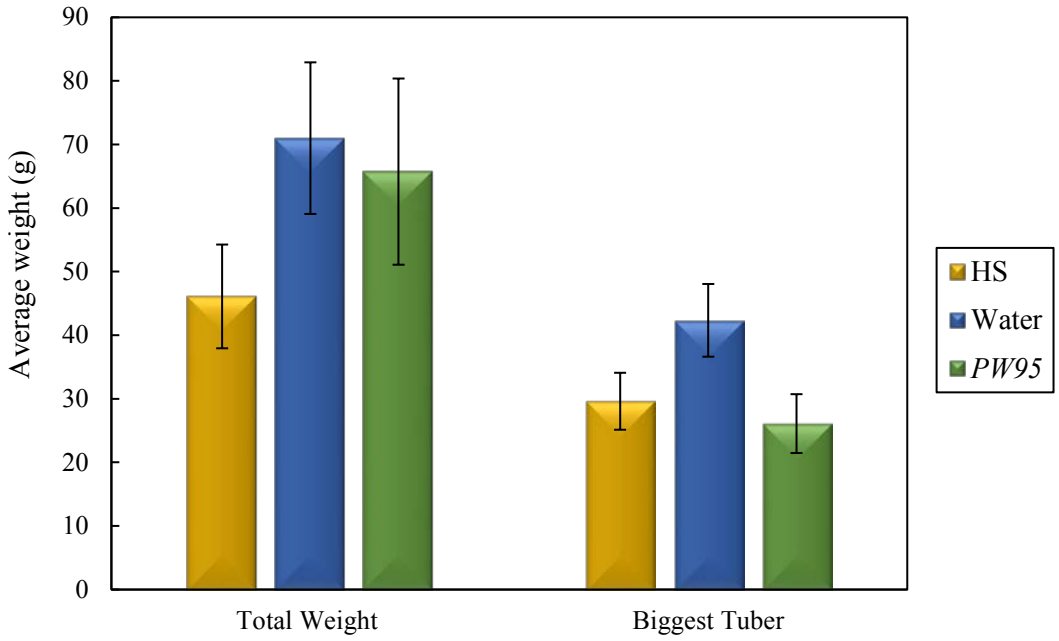

Figure 12: Potato Harvest Yield

4.1.3. Flax

Linum usitatissimum plants grown in one liter pots were treated with *PW95*, *A. cylindrica*, Hoagland solution, and water. Chlorophyll data indicated not much difference amongst the four treatments in the first 70 days of development. However, measurements on day 115 (Figure 13) showed that *A. cylindrica* -treated flax had about 21% and 23% more chlorophyll content than *PW95*- and Hoagland solution - treated Flax plants. This result is suspected to be due to the very high sensitivity of Flax plants to alkaline conditions (Duke, 1983).

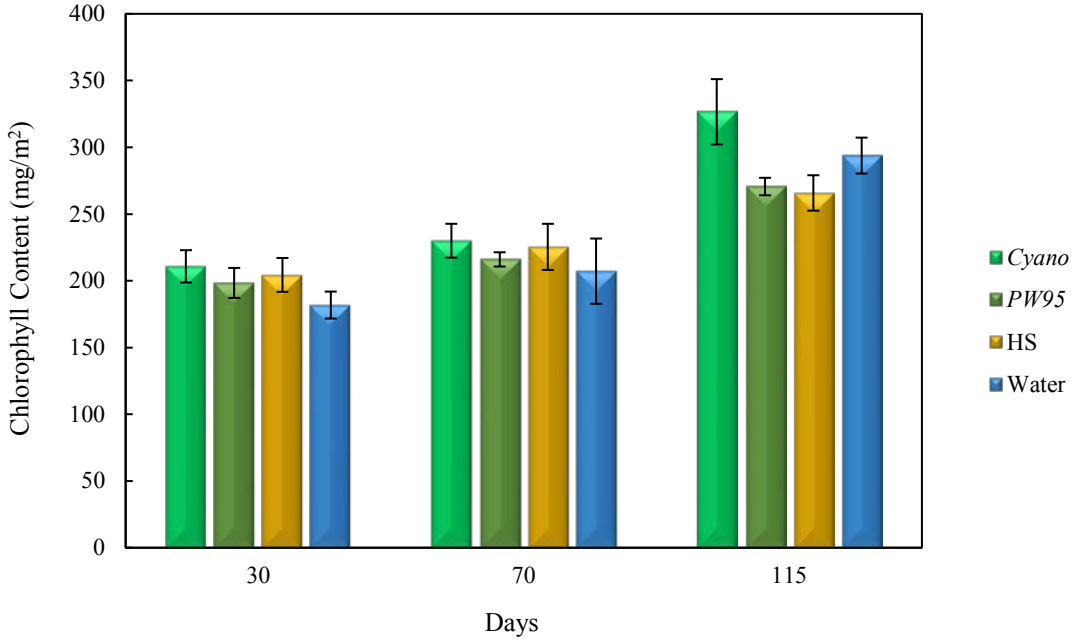

Figure 13: Flax Chlorophyll Measurements

4.2. *PW95* - Induced Flocculation

The effectiveness of flocculation induced by 0.1 M KOH was tested in replicates at four different pH levels of 10, 11, 11.5, and 12 (Figure 14). The 11.5 pH level was included due to the result of a preliminary experiment that indicated an optimum pH between 11 and 12. This observation agrees with studies conducted by Blanchemain *et al*. (1994), and Yahi *et al*. (1994). The initial pH, absorbance, and turbidity of the *PW95* medium were measured as 9.5, 0.385 A, and 42.1% respectively (Appendix D). It was deduced that the flocculation efficiency is directly related to absorbance and turbidity values. Likewise, the efficiency is also affected by cell density and the settling. As was previously indicated in Chapter 3, 50 mL of the suspension (2.75 x 10^6 cells/mL) were used for the experiment. Determination of the dry weight of *PW95* gave a value of 1.0 g/L.

Figure 14: PW95 suspension after KOH addition

Figure 15 is a plot of the volume of 0.1M KOH used to raise the pH levels. It was evident that more KOH was required to raise the pH levels. The least 0.1 M KOH volume of 0.1 mL (0.6 mg) was used in raising the pH from 9.5 to 10 while 2.4 mL (13.5 mg) was used in raising the pH from 11.5 to 12. Using the mass of KOH (5.0 mg) required to raise suspension pH to 11.5, it could be deduced that 3,600 kg of KOH to flocculate the Rancholme 4-34 (Doc Holiday 2) pond shown in Table III.

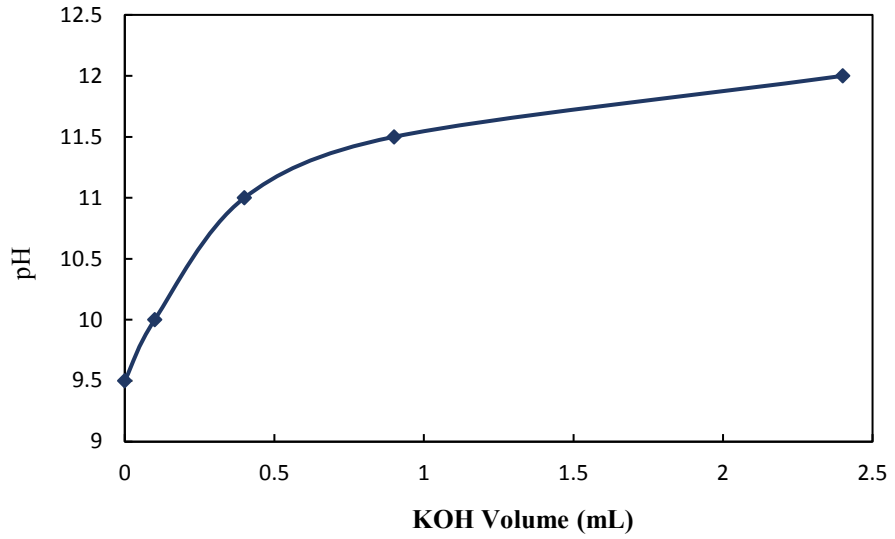

Figure 15: pH vs KOH Volume (mL)

In Figure 16, a relationship was observed between flocculation efficiency and settling time with respect to pH. This relationship can be attributed to cell aggregation which would conversely increase the rate of settling. Highest change in flocculation efficiencies of 28% to 42% were achieved at pH 11.5 over a settling time of 15 and 30 minutes. At 45 minutes, the flocculation efficiency at pH 12 increase to 51.82%. This increase was 3.4% higher than the efficiency recorded for pH 11.5 at 45 minutes.

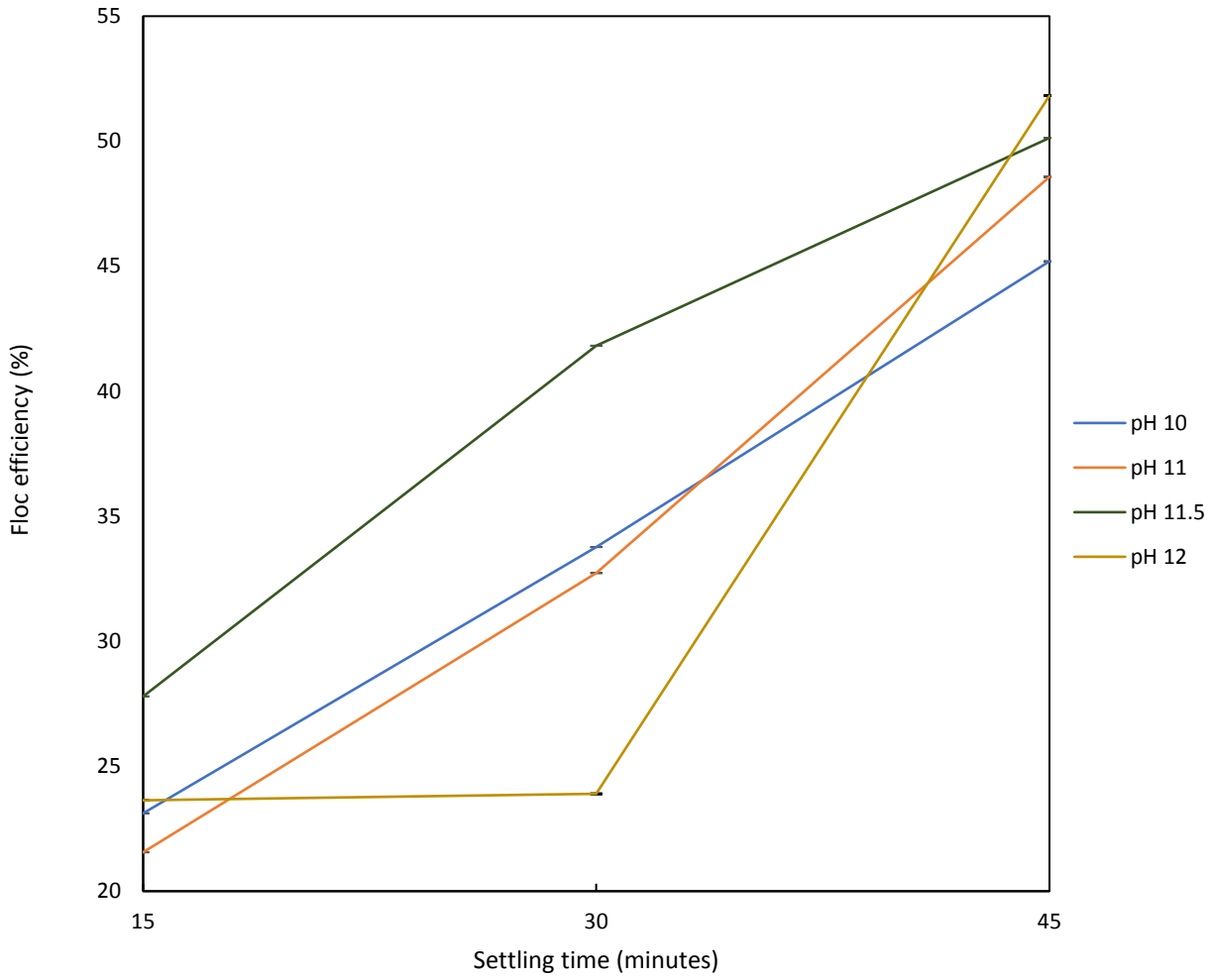

Figure 16: Floc efficiency vs settling time

A correlation existed between absorbance and pH with respect to settling time (Figure 17). Absorbance measurements become lower as settling time increase except for pH 12 at settling times of 15 and 30 minutes where there were spikes in absorbance readings. Similar to Figure 16, lowest absorbance levels were measured for pH 11.5 at settling times of 15 and 30 minutes.

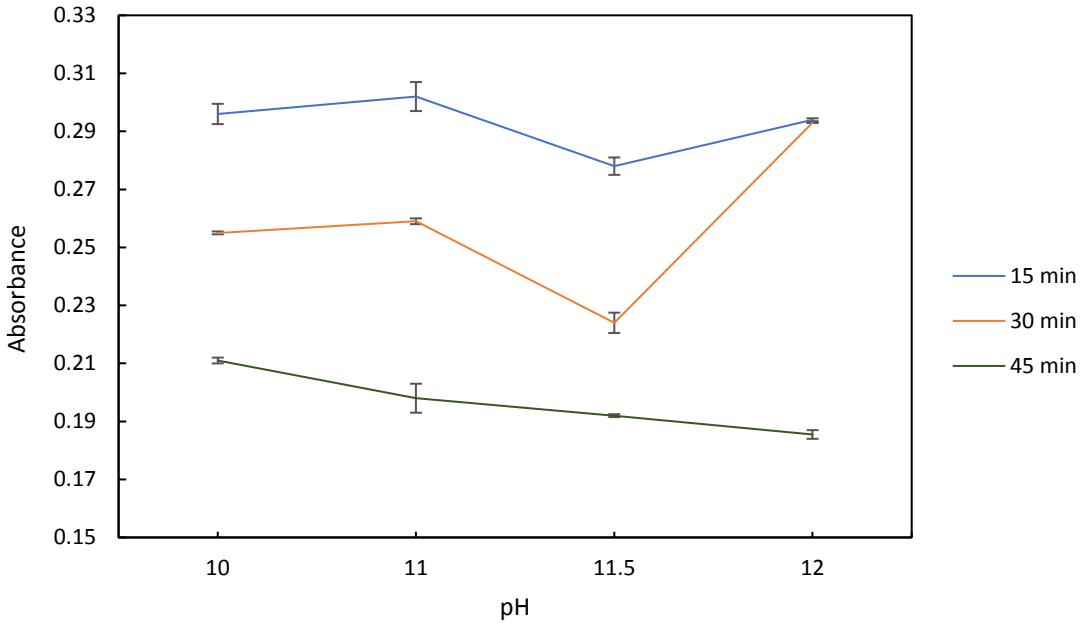

Figure 17: Absorbance vs pH at different settling times

5. Conclusions

The result of the fertilization experiment affirmed that microalgae have essential micro- and macro-nutrients that can be deployed as biofertilizer leading to accelerated production of vital economical crops like winter wheat (*Triticum aestivum*). Result of the nutrient analysis for *PW95* clearly signposted nitrogen (1240 ppm) as the most available of all the primary macronutrients - well ahead of potassium (264 ppm) and phosphorus (130 ppm). The result also indicated the presence of secondary and tertiary macronutrients (sulfur, calcium, and magnesium) and some micronutrients - trace minerals - such as sodium, iron, aluminum, zinc, manganese, and boron.

The *PW95*-fertilized wheat consistently showed higher values for all the essential growth indicators (chlorophyll content, number of tillers, and flowers) when compared to wheat fertilized by Miracle-Gro and water. An analysis of harvest data showed that *PW95*-fertilized wheat had the best result in terms of total 'above-ground' weight and seed weight. Specifically, the total 'above-ground 'weight for wheat fertilized by *PW95* was 117% and 47% higher than was measured for wheat fertilized by water and M.Gro respectively. Also, measurements of the seed weights indicated that *PW95*-fertilized wheat was 123% and 58% more than corresponding measurements for those fertilized with water and M.Gro respectively.

Similar fertilization investigations were conducted on Ranger Russet seed potato (*Solanum tuberosum*) and Flax (*Linum usitatissimum*) crops using *PW95* and nitrogen-fixing *Anabaena cylindrica* as the fertilizing agent. However, the resulting harvest data are not comparable to those obtained for wheat fertilization experiments as the water-fertilized potatoes (control group) produced heaviest tubers. Consequently, two hypotheses were considered as the basis for the low harvest for potatoes fertilized by *PW95*, *A. cylindrica*, and chemical fertilizer.

One of such postulates suggest that the result might be cause by excess nitrogen made available by the fertilizing agents (*PW95*, *A. cylindrica*, and chemical fertilizer). This hypothesis was based on the common knowledge that root crops do not require huge application of nitrogen as it only results in the development of potato plant with poor tuber growth. The second theory simply considered the possibility that microalgae might only be suitable for monocots than dicots.

The flocculation experiment verified the effectiveness of 0.1 M KOH in inducing aggregation and settling of *PW95* cells. Highest flocculation efficiencies (FE) were observed at optima pH 11.5 to 12. The lowest effective flocculant dosage (5 mg KOH) was observed at pH 11.5 while the maximum dosage (13.5 mg KOH) was observed at pH 12. As stated in literature, dominant flocculation induced by alkaline flocculants is through the neutralization of the repelling surface charge of algae cells. As observed from the plots of flocculation experimental data, there is a direct relationship between FE and settling time with respect to pH. This relationship confirms that increased formation of microalgal flocs at high pH can lead to increased rate of settling. Possible concerns with the use of biomass slurry from the flocculation experiment might be land contamination and the effect of high pH on crops. As stated by Goyal & Gimmler (1989), increase in pH of the medium does not necessarily correlate to an increase of pH within the algal cell. Accordingly, the best application procedure would involve further concentration of the microalgae through drying. Alternatively, the alkaline biomass can be used to fertilize edible and ornamental crops that thrive well in alkaline conditions.

Fossil fuels, including coal, oil and natural gas, are currently the world's primary energy source. The exploration of these resources and their uses is known to have many negative impacts on the environment. One of such great consequence originates from the production processes involving the intensive input of clean water and output of highly polluted water which

can compromise the ecosystems and other water usage such as food production. Coal Bed Methane produced water is a waste product from the activities of such production processes. The conservation of freshwater resources is desirable in today's world. Hence, the results of this study have shown that the enormous volume of the CBM produced water can be a great resource thereby limiting the growing constraints on the availability, quality, and use of freshwater resources.

Likewise, with the increasing global demand for food and water, various methods and techniques are being evaluated in a bid to increase food production. Great dependency has been placed on the use of chemical fertilizers to increase food production. However, the prices of this reliance are all too evident in contamination of water sources and agricultural produce. This study has shown that microalgae has the great potential in accelerating crop yields of agricultural produce. Unlike chemical fertilizers that break up soil structure over time, microalgae are known to be effective soil conditioners by facilitating the gradual buildup of residual soil nitrogen and carbon, and improving soil pH and electrical conductivity without any ecological degradation. Hence, the use of microalgae as biofertilizer will not only reduce fertilizer cost for Montana farmers, it could also lead to a reduction in ecosystem contamination resulting from runoff from fields fertilized by chemical fertilizers.

This study is presently ongoing and has been expanded to investigate the impact of *A. cylindrica* biofilm on wheat by measuring soil geochemistry parameters such as soil moisture, temperature, and electrical conductivity (Appendix G: Figure 21). Also, analysis of nutrient composition is ongoing. Future works would involve the utilization of algal biomass cultured in CBM water and flocculated by KOH as biofertilizer. Upon availability, biomass from CO_2 air-capture process would also be investigated for fertilization potentials. At the end of these

experiments, assessment of the agricultural produce would be conducted to determine if they are fit for consumption.

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7. Appendix A: Potato Chlorophyll Content and Harvest Data

Table J: *PW95*-Fertilized Potatoes Harvest Data

Plant	W_i (g)	W_i Biggest (g)	W_D (g)	W_D Biggest (g)	# Tubers
P 1	61.153	53.247	50.34	14.811	6
P 2	101.195	34.963	82.485	29.815	9
P 3	25.813	18.973	21.708	16.11	2
P 4	38.109	18.019	32.807	15.49	3
P 5	135.175	17.37	96.234	38.166	7
P 6	25.163	15.333	22.412	14.176	4
P 7	21.799	11.101	17.526	8.872	4
P 8	139.866	30.93	113.865	26.57	7
P 9	27.007	14.456	19.786	10.184	4
P 10	82.053	46.612	65.24	39.162	5
HS 1	39.108	39.108	37.121	37.121	1
HS 2	22.425	16.377	19.868	14.436	2
HS 3	36.649	25.076	28.434	18.895	2
HS 4	65.139	25.277	53.09	20.604	4
HS 5	14.713	10.606	12.211	8.944	2
HS 6	40.219	24.399	33.764	19.685	2
HS 7	99.15	61.33	79.538	48.189	3
HS 8	30.272	25.589	26.404	22.48	2
HS 9	74.297	29.318	61.842	25.265	5
HS 10	39.075	39.075	35.408	35.408	1
W 1	22.038	22.038	18.412	18.412	1
W 2	39.948	31.455	33.87	26.321	3
W 3	77.189	47.077	64.368	40.449	5
W 4	34.478	30.212	30.931	27.188	2
W 5	151	57.541	126.464	49.364	7
W 6	93.348	25.567	76.894	22.086	7
W 7	70.054	49.137	60.732	43.063	2
W 8	70.905	35.348	57.517	28.426	5
W 9	99.143	82.786	87.18	73.638	2
W 10	51.881	42.273	44.964	37.678	3

P - PW95 HS – Hoagland solution W – Water

Table K: Chlorophyll Content measurements (mg/m^2)

	Day 60			Day 88		
s/n	**HS**	**Water**	***PW95***	**HS**	**Water**	***PW95***
1	217	186	224	148	116	205
2	211	167	243	173	122	160
3	211	160	217	198	179	186
4	173	211	255	154	116	192
5	217	217	249	205	160	173
6	243	236	224	186	179	148
7	224	249	211	160	160	255
8	243	192	243	91	193	173
9	217	167	224	179	179	205
10	236	198	230	179	165	192

P - PW95 HS – Hoagland solution W - Water

9. Appendix B: Wheat Height and Harvest Data

Table L: Wheat Height Data (cm)

	Day 7			Day 13			Day 33			Day 67		
s/n	**M. Gro**	**Water**	***PW95***	**M. Gro**	**Water**	***PW95***	**M. Gro**	**Water**	***PW95***	**M. Gro**	**Water**	***PW95***
1	4.50	8.20	7.00	23.10	22.00	23.50	52.07	38.74	47.63	81.92	54.61	73.03
2	3.70	7.50	8.60	21.60	24.00	19.60	41.28	44.13	48.26	83.19	57.15	71.12
3	5.40	7.40	6.50	21.50	21.00	19.00	45.72	36.83	45.72	71.76	49.53	73.66
4	8.20	7.00	6.10	24.10	23.60	20.00	50.80	39.69	43.18	81.28	55.88	63.50
5	7.00	4.50	7.60	20.20	23.00	22.10	50.17	41.28	43.82	81.92	49.28	63.50
6	7.00	7.00	9.50	20.10	22.00	22.30	46.36	40.64	50.80	80.01	62.87	67.31
7	8.10	5.95	9.20	22.80	22.90	17.00	45.72	36.83	48.90	83.82	49.53	58.42
8	6.70	6.70	8.70	16.70	24.00	17.70	50.80	48.58	43.18	87.63	55.88	78.74
9	5.50	7.70	9.85	20.80	22.30	20.00	52.07	39.69	47.63	89.54	58.42	77.47
10	6.00	7.50	5.75	20.60	22.10	20.20	52.71	50.17	50.17	72.39	65.41	80.01
11	6.90	5.80	7.70	20.20	21.00	23.30	52.07	46.36	43.18	67.31	67.31	69.22
12	7.70	7.60	7.40	24.00	20.05	21.10	41.91	41.91	43.18	78.74	62.87	74.93
13	5.30	7.50	7.75	18.30	20.80	20.40	52.07	42.86	48.26	85.09	54.10	80.01
14	6.70	9.60	9.00	12.00	22.30	22.30	45.09	46.99	43.18	83.19	69.22	57.15
15	8.20	6.30	8.80	25.00	21.10	20.20	56.52	47.63	40.64	63.50	60.96	73.66
16	7.60	7.60	7.10	22.00	21.10	19.70	46.99	42.55	50.80	77.47	59.69	60.96
17	7.80	7.40	8.10	21.80	22.20	17.00	50.80	40.64	48.26	80.01	88.90	85.09
	Day 7			**Day 13**			**Day 33**			**Day 67**		

s/n	M. Gro	Water	*PW95*	M. Gro	Water	*PW95*	M. Gro	Water	*PW95*	M. Gro	Water	*PW95*
18	7.40	7.00	9.50	20.10	22.90	16.50	53.34	43.82	43.18	74.93	71.83	52.07
19	4.60	8.10	8.30	23.00	24.10	21.50	42.55	45.09	48.90	79.38	62.87	52.71
20	6.00	8.80	7.80	21.20	18.50	22.00	48.77	39.37	51.44	73.66	71.12	66.04
21	8.55	6.20	9.50	24.50	22.80	21.60	45.09	46.99	41.72	80.65	54.36	60.96
22	8.25	9.00	8.40	21.40	21.00	23.30	44.45	45.72	44.45	71.76	75.57	69.22
23	8.30	7.60	9.00	22.90	23.70	21.00	46.99	40.64	41.91	85.09	67.31	59.69
24	7.10	8.20	9.50	23.30	22.80	19.00	45.72	44.45	50.48	73.66	66.04	64.77
25	8.00	7.10	9.50	22.10	24.30	20.50	43.82	42.55	45.72	76.84	63.50	68.58
26	6.90	7.40	5.20	22.60	16.80	22.10	40.64	40.32	46.36	77.47	52.71	53.34
27	5.70	6.40	8.55	20.40	15.20	17.30	39.37	43.18	43.18	71.76	40.64	82.55
28	8.50	5.80	8.50	22.50	18.50	16.00	41.91	41.91	52.07	83.82	44.45	65.41
29	1.50	2.00	9.10	11.60	21.30	15.50	48.26	33.02	46.04	71.76	70.49	63.50
30	6.70	6.50	8.20	21.00	21.00	17.70	45.72	38.74	50.17	76.84	53.34	56.52

Table M: Wheat Harvest Data

	Total Dry Weight			Seed Dry Weight			Flowers		
s/n	**M. Gro**	**Water**	***PW95***	**M. Gro**	**Water**	***PW95***	**M. Gro**	**Water**	***PW95***
1	2.5	0.6	1.9	1.5	0.3	1	2	1	2
2	1.2	0.9	5.1	0.5	0.6	2.9	1	1	4
3	1.8	1.2	3.1	1.5	0.7	1.8	1	2	3
4	3.6	5.8	3.8	1.9	3.1	2.4	3	5	3
5	1.8	0.6	4.3	1	0.3	2.4	2	1	4
6	2.6	1.8	3	1.3	1	1.8	2	3	3
7	3.7	1	3	1.9	0.6	1.8	4	2	2
8	1.4	2.1	4.7	0.8	1.1	2.9	2	3	3
9	2.9	2	5	1.7	1.2	3	3	2	3
10	2.1	1.5	4.7	1.1	0.9	2.7	2	2	4
11	3.8	3.5	2.2	2.3	1.9	1.3	4	4	2
12	2.7	7.1	8.3	1.5	4.1	5	2	6	5
13	3.4	1.9	6.7	1.8	1.1	3.9	3	2	5
14	3.3	0.9	1.3	1.8	0.5	0.6	3	2	1
15	2.1	1.6	3.5	1.2	0.9	2	3	3	3
16	3.3	1.2	2.9	1.8	0.6	1.7	3	2	3
17	2	2.5	4.3	1.2	1.5	2.3	2	3	4
18	4.1	2.8	3.5	2.4	1.5	2	3	3	4
19	4.7	1.2	5.8	2.5	0.7	3.5	3	2	5
20	3.4	1.3	5.6	1.6	0.8	3.6	3	2	3
21	3.2	1.8	4	1.4	1	2.3	3	3	4
22	4	1.3	5	2.2	0.8	3.2	4	2	4
23	3.2	2.1	5.9	1.7	1.1	3.5	3	3	5
24	2.4	1.6	2.3	1.4	0.9	1.2	2	3	2
25	3.4	1.3	2.8	1.8	0.8	1.6	3	2	2
26	3	0.7	4.1	1.6	0.4	2.6	5	2	3
27	2.7	1.1	7.1	1.5	0.6	3.9	3	2	5
28	3.3	2.1	2	1.8	1.2	1.1	2	3	2
29	3.3	3.4	6.2	1.8	2.1	3.6	4	3	5
30	1.7	1.9	5.5	0.9	1.2	3.2	2	2	6

10. Appendix C: Flax Fertilization Data

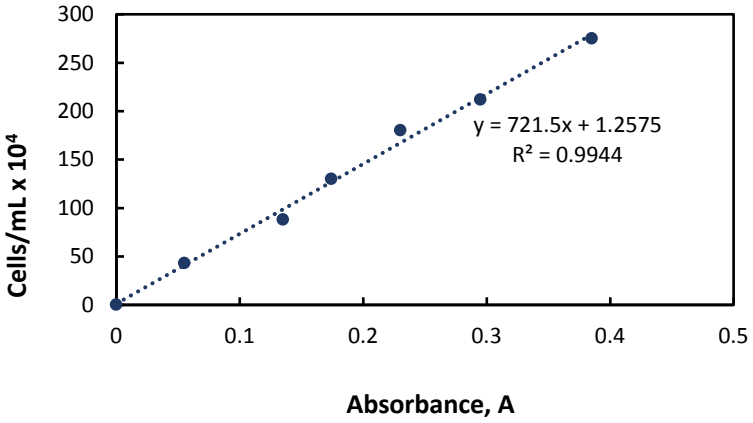
Table N: Flax Fertilization Data

Pots #	Day 30				Day 70				Day 115			
	Cyano	*PW95*	HS	Water	Cyano	*PW95*	HS	Water	Cyano	*PW95*	HS	Water
1	186	192	160	198	230	217	236	217	389	268	300	294
2	236	192	211	173	230	205	230	230	249	249	249	243
3	179	236	217	154	275	236	281	262	363	281	275	313
4	236	205	198	211	198	211	179	116	332	268	224	300
5	217	167	236	173	217	211	201	211	300	287	281	319

11. Appendix D: Flocculation Data

Table O: *PW95* Flocculation Data

Cell Count/mL	2,750,000
Suspension pH	9.5
Suspension OD	42.1
Suspension AB	0.385
KOH Conc. [M]	0.1
Sample volume, mL	50
Dry weight, g/L	1.0 ± 0.1



pH	KOH Volume (mL)	AB 15	AB 30	AB 45	OD 15	OD 30	OD 45
10	0.1	0.30	0.26	0.21	50.60	55.60	61.60
		0.29	0.25	0.21	50.80	55.70	61.70
11	0.4	0.31	0.26	0.20	49.50	54.90	62.70
		0.30	0.26	0.19	50.50	55.00	64.00
11.5	0.9	0.28	0.23	0.19	52.40	59.30	64.20
		0.28	0.22	0.19	53.00	60.50	64.30
12	2.4	0.29	0.29	0.18	51.80	52.00	65.50
		0.29	0.29	0.19	51.70	51.80	65.90

						Absorbance		Cell Count		Floc Efficiency	
pH	**KOH Volume (mL)**	**KOH mass (mg)**	**15**	**30**	**45**	**15**	**30**	**45**	**15**	**30**	**45**
10	0.1	0.6	0.30	0.26	0.21	214.82	185.24	153.49	23.12	33.77	45.19
11	0.4	2.2	0.30	0.26	0.20	219.15	188.13	144.11	21.56	32.73	48.57
11.5	0.9	5.0	0.28	0.22	0.19	201.83	162.87	139.79	27.79	41.82	50.13
12	2.4	13.5	0.29	0.29	0.19	213.38	212.66	135.10	23.64	23.90	51.82

12. Appendix E: *PW95* Algal cells

Figure 18: *PW95* Algal cells

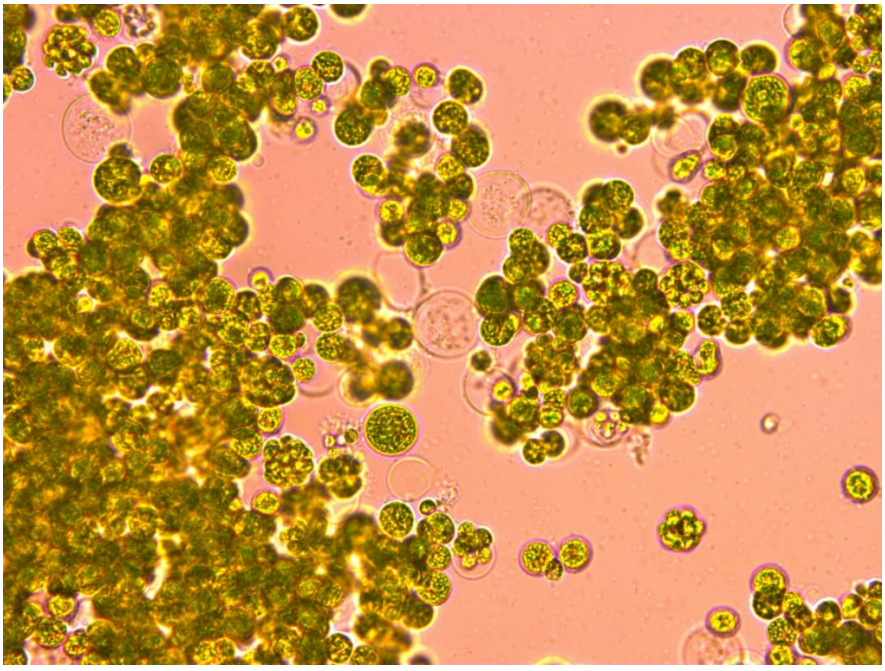


Figure 19: Flocculated *PW95* cells

13. Appendix F: *A. cylindrica* cells

Figure 20: *A. cylindrica* cells

14. Appendix G: Ongoing Research

Figure 21: Ongoing Research – *A. cylindrica* Biofilm Investigation

15. Appendix H: Material Safety Data Sheet for Soil

Sunshine #4 Bale

Material Safety Data Sheet

SUN GRO HORTICULTURE

Date Reviewed and Issued: March 1, 2008 **MSDS#** #1000

Section 1. Identification: Professional Growing Media- Peat Based

Product Names:

- Sunshine LC1 and #1
- Sunshine LB2 and #2
- Sunshine LG3 and #3
- Sunshine Mezcla Mix #3
- Sunshine LG3 XV
- Sunshine LA4 and #4
- Sunshine LA4V
- Sunshine LP5 and #5
- Sunshine LT5
- Sunshine LPM6 and #6
- Sunshine GP7 and #7
- Sunshine LC8 and #8
- Sunshine Growers Germination
- Sunshine Pansy
- SC1
- SC2
- Sunshine EuroBlend
- Sunshine Special Blend
- Sunshine Custom Blend

Manufacturer/Supplier:
Sun Gro Horticulture Canada Ltd.
P.O. Box 189
Seba Beach, AB T0E 2B0

For more information call:
Western Region 1-888-797-7328
Central Region 1-888-982-4500
Eastern Region 1-888-896-1222
For more information: www.sungro.com

Product Uses: Horticulture

Section 2. Hazardous Ingredient & Composition

		OSHA PEL		ACGIH TLV	
Component	**CAS#**	**Total**	**Respirable**	**Total**	**Respirable**
Nuisance dust	Not Applicable	15 mg/m3	5 mg/m3	10 mg/m3	3 mg/m3

Comments: This product is not considered to be hazardous in accordance with the OSHA Hazard Communication Standard 29 CFR 1910.1200, Health Hazard. The product is classified by ACGIH as a "Nuisance Dust" or "Particulates Not Otherwise Classified" (PNOC). See product label for ingredients and product specific information.

Section 3. Physical/Chemical Characteristics

Physical State:	Solid	**Boiling Point:**	Not applicable
Appearance:	Granular, soil-like	**Vapor Pressure:**	Not applicable
Color:	Brown, earthy color	**Solubility in Water:**	Not applicable
Odor:	Earthy	**Melting point:**	Not applicable
pH:	3.5 to 7.5	**Evaporation rate:**	Not applicable
Density:	100-200 g/l or 6-12 lb/cubic foot	**Specific gravity:**	Not applicable

Section 4. Fire and Explosion Hazard Data

Flash Point:	Not Applicable
Flammable Limits:	Not Applicable
Extinguishing Media:	Water, fog or spray
Special Fire Fighting Procedures:	None. Caution, burning may continue inside bags or piles after surface fire is out. Break bags or separate pile to assure that the fire is extinguished. The thermal decomposition products are those commonly observed with natural products such as wood or other vegetative matter.
Unusual Fire and Explosion Hazards:	None

Section 5. Stability and Reactivity

Stability:	Stable
Conditions to Avoid:	See label. Keep away from heat, sparks and open flames.
Incompatibility:	None known
Hazardous Decomposition or Byproducts:	High temperatures or fire may produce irritating gases and vapors.
Polymerization:	Will not occur
Incompatible Materials:	None known

Section 6. Health Hazard Data

Routes of Entry:	Inhalation, open wounds and eyes
Health Hazards:	Nuisance dust. See product label for ingredients
Carcinogenity:	None known
Effects of Exposure:	Inhalation over long periods of high amounts of any nuisance dust may overload lung clearance mechanism, irritate mucous membranes and make lungs more vulnerable to respiratory disease.
Emergency and First Aid Procedures:	If inhaled, provide fresh air. If eye irritation occurs, flush with water. Keep open wounds covered and clean as suggested by any good program of hygiene.
Other Concerns:	Keep out of reach of children and pets.

Section 7. Toxicological Information

General Comments: Inhalation of dust may irritate nose, throat and lungs. Eye contact with solids may produce irritation, tearing or blinking as a foreign body in the eye.

Section 8. Precautions for Safe Handling and Use

Steps to be taken in case material is spilled:	Use methods to clean spill which avoid creating airborne dust.
Waste Disposal Method:	According to EPA 40 CFR 261.3, waste of this product is not defined as hazardous. Dispose of all waste in accordance with federal, state and local regulations.
Precautions to be taken in handling and storage:	If excessive dust is created avoid breathing dust by using adequate ventilation and/or using NIOSH or MSHA approved respirator for nuisance dust of this type. Breathing dust may be harmful to your health.
Other Precautions:	Protective eyewear should be worn where dust levels are high enough to cause irritation.
Ecological Information:	Keep out of lakes, streams or ponds.

Section 9. Control Measures

Respiratory Protection:	If dust is created use NIOSH or MSHA approved respirator for nuisance dust of this type.
Ventilation:	Local exhaust advisable if excessive dust is created.
Protective Gloves:	Not normally necessary but suggested in cases of open wounds that are not appropriately protected.
Eye Protection:	Protective eyewear should be worn where dust levels are high enough to cause irritation.
Other Protective Clothing or Equipment:	Normal work clothing
Work/Hygienic Practices:	NIOSH or MSHA approved respirator, eye protection and ventilation under conditions where excessive dust is created. Open wounds should be kept clean and suitably protected.

Section 10. Supplemental Information

These materials are made from natural products and may contain naturally occurring microorganisms. Proper precautions are advised to prevent infection of open wounds, inhalation of excessive amounts of dust and eye irritation. The proper hygiene practices necessary to prevent health hazards from any naturally occurring substance such as soil, bark, etc., should be observed.

SIGNATURE PAGE

This is to certify that the thesis prepared by Olakunle Ogunsakin entitled "EVALUATION OF THE FERTILIZATION PROPERTIES OF MICROALGAL BIOMASS AND ASSESSMENT OF KOH-INDUCED FLOCCULATION OF PW95 ALGAL CELLS" has been examined and approved for acceptance by the Department of Environmental Engineering, Montana Tech of The University of Montana, on this 21st day of June, 2017.

Kumar Ganesan, PhD, Professor
Department of Environmental Engineering
Chair, Examination Committee

Martha Apple, PhD, Professor
Department of Biological Sciences
Member, Examination Committee

Bill Drury, PhD, Professor
Department of Environmental Engineering
Member, Examination Committee

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